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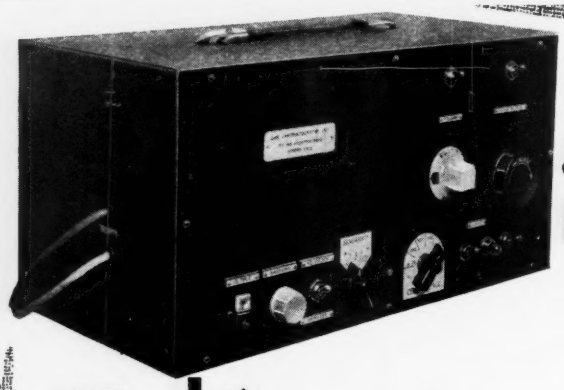

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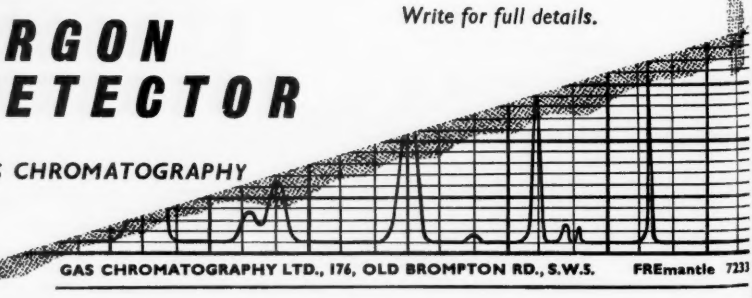

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EXPANSION OF UNIVERSITY EDUCATION IN BRITAIN

IN an address to the eighth Congress of the Universities of the Commonwealth* at Montreal in September 1958, Sir Douglas Logan, principal of the University of London, opening a discussion on the establishment of new universities, expressed the opinion that in all parts of the world there is to-day a widespread demand for an expansion of the facilities for higher education which can be satisfied only by the creation of new universities. While he paid tribute to the way in which the scheme of 'special relation' had contributed to the growth in the post-war period of the new university colleges in the overseas Commonwealth, he pointed out that the scheme is costly both in time and man-power, making heavy demands on the university foster-mother. He believes, too, that in England we have reached the end of an era when apprenticeship under the London external degree system was the avenue by which an institution approached full university status, but nevertheless we are reaching the limit of expansion of our existing universities, and the problem of expanding facilities for higher education in the United Kingdom can, in his opinion, only be solved by the establishment of new university institutions.

Sir Douglas Logan believes that in this matter the initiative should come from outside, and that it is disappointing that the new University College of Sussex would only be the fifth to be founded in the United Kingdom since the end of the First World War. The founding of other university institutions is, of course, already being actively canvassed, for example, at York, at Norwich and at Bournemouth, and it will at once be realized that many aspects of long-term university policy are implicit in Sir Douglas's address. Apart from the question of where new university institutions should be established and how many, there are such questions as the size of a university, new or existing, what type or types of new institution should be established, the optimum size of its departments and faculties, their range of studies and, probably, the length of their courses. Nor can these questions be wisely considered without regard to Britain's existing resources, the desirable output of graduate and postgraduate students and the cover of faculties.

These questions have been the subject of increasing discussion in recent months. Early this year in *The New Scientist*, Prof. W. G. V. Balchin, of Swansea, argued that universities are a product of population and have risen in response to national, civic and regional needs. A new set of regional university colleges is desirable, he believes, to satisfy the future demand for places, to maintain the standards of existing institutions and permit experimentation. The belief is widely held that the optimum size of a university is between 3,000 and 4,000 students, but there is also strong support for smaller units of 2,000 to 2,500. Prof. Balchin listed a dozen or more regional centres for consideration where preliminary moves have been made.

The decision as to the number of new institutions must be determined by the desirable target for national output, as well as by the views held as to the appropriate size of individual institutions. The University Grants Committee has given a figure of 136,000 as a suitable objective for Britain before the end of 1960. The Association of University Teachers, however, estimates that the university population should rise to 176,000 by 1960, falling to about 145,000 a few years later. Present Government grants based on the first figure are expected to provide an annual output of 12,000 science and engineering graduates by 1970, and with 10,500 qualified men from the technical colleges, will increase the student-population ratio to something like 1 : 410.

To a large extent the argument for university expansion in Great Britain rests on the alleged need for more scientists and technologists, and the analysis of this need by Prof. John Jewkes in his presidential address to Section F (Economics) at the British Association meeting at York is accordingly highly important in assessing the desirable magnitude of university expansion as a whole. Inevitably, the striking of a right balance between the scale of scientific and technical activities and other activities is a matter of judgment, perhaps also of intuition. Prof. Jewkes suggests, in fact, that our knowledge about our scientific and technical effort is really very limited, and that we know little about even the true magnitude of the needs of Britain in man-power.

Prof. Jewkes does not believe that the universities of Britain are at present failing in their task of selection, and accordingly if more people are trained in them in the sciences and in technology, fewer of the ablest segment of the community will be trained in the other intellectual disciplines. This could be the more serious if there is, in fact, not simply in the undeveloped parts of the world, but also elsewhere, a growing need for more men of the administrative and managerial type, and for the pervasive spread of specialized skills of all kinds which Western countries take for granted. Indeed, important as it is that the administrator and manager should be aware of scientific and technical factors in a situation and able to assess their importance, it could equally be argued that the growing need of to-day is for the imaginative insight, the vision and capacity to assess human values that a humanistic education can inculcate—a view which President Eisenhower's

* Eighth Congress of the Universities of the Commonwealth, Montreal, September 1–5, 1958: Report of Proceedings. Pp. xxix + 100. (London: Association of Universities of the British Commonwealth, 1959.) 12*s.* 6*d.*

Science Advisory Committee appears to share in its statement, "Education for the Age of Science".

Here, indeed, the question of quality is all-important, and the possibility and consequences of still further increasing the proportion of failures (already higher in science and technology than in other subjects) in a policy of university expansion in which standards are not lowered must be considered. Here, too, it should be remembered that the decisive contribution to economic or industrial expansion and to scientific advance comes from the comparatively few outstanding and creative minds. The right question is undoubtedly, as Prof. Jewkes suggests, whether the community will gain by having more scientists and more technologists trained at the expense of non-scientists; but this too is linked with the question of quality and whether, in fact, we are at present employing our scientists and technologists to the best advantage and, in particular, providing them with the technicians and other assistance as well as equipment which are required to make the most efficient use of their services. Nor, as Prof. Jewkes also observes, can we overlook the competition which exists for the services of scientists, for example, between the need for teachers of science and scientists in industry or government service.

Prof. Jewkes is inclined to challenge the assumption that Great Britain is suffering from an unmet need for more scientists, and he is highly critical of the evidence or criteria on which the argument for such a shortage has been based. Neither international comparisons nor the attempts to correlate industrial output and the number of scientists and technologists are convincing; nor again does it appear reasonable to assume that, in proportion to size, every industry should spend the same proportion on research and development or devote the same proportion of its labour force to those ends. Measurement of the net gain arising from research and development is equally difficult, and there seems to be no strong evidence that the appearance of modern industrial research laboratories about 1930 has made any steeper the general upward trend in output per head. Again, comparing experiences of the different industrial countries since 1945 provides no very obvious correlation between the rate of industrial expansion and the resources devoted to scientific and technological effort.

Prof. Jewkes suggests, therefore, that we should continue to search for better criteria for determining how much the community should devote to science and technology, but points out that meanwhile the striking of a correct balance is not in itself a scientific process or one in which scientists should have the last word. He reminds us, moreover, that social discoveries in their totality may have contributed just as much to the rise of standards of living in the past two centuries as the technical inventions and scientific discoveries of the period. He does not suggest that we do not need more scientists and technologists; he does suggest that we should be clear as to the full implications of such expansion both in the universities and elsewhere, before we commit ourselves to a policy of expansion on any particular scale.

Lord Simon, in an article on university expansion in the *Universities Quarterly* of 1958–59 (**13**, No. 1), argued strongly for the establishment of a Royal Commission on the Universities to examine both university expansion and long-term university policy. He claimed that long-term university policy is scarcely considered by the University Grants Committee and that the Committee's very success in dealing with heavy and vitally important administrative matters leaves it little or no time to devote to matters of long-term policy. Lord Simon instanced many matters which such a body might consider, such as the question of halls of residence and the extent to which new universities should be primarily residential, and the relations between teaching staff and students, but without advancing convincing reasons why such matters should be considered by such a body as a Royal Commission rather than some appropriate committee appointed by the University Grants Committee itself.

There is, of course, involved here the fundamental question which Minister or Department of State should primarily be charged with university policy. Besides the Chancellor of the Exchequer, to whom the University Grants Committee is responsible, the Minister of Education and the Lord President of the Council are both involved; and Lord Hailsham has already made it clear that he regards university policy as to some extent falling within his new responsibility for fresh thinking about the whole structure of Britain's scientific and technical effort. This follows inevitably from his responsibilities for the Department of Scientific and Industrial Research. Such really prickly questions as the magnitude of the alleged distorting effects of the prestige and resources of the older universities in comparison with the new ones, and the implications and consequences of requiring a greater expansion in the scientific departments than academic circles regard as healthy for university life might well be handled by him without resort to the expedient of a Royal Commission, with its demands on men and women whose judgment, vision, authority and wide experience are already heavily engaged.

The question of a Royal Commission has again been raised by G. C. Moodie in a pamphlet issued by the Fabian Society. In "The Universities: a Royal Commission?"*, Mr. Moodie sets forth eighty-eight questions for consideration by such a commission without, however, seriously examining the justification for appointing a Royal Commission to consider all these matters, some of which are clearly comparatively trivial and others which Mr. Moodie himself admits could not usefully be examined by a purely United Kingdom Commission, since any action would necessarily have to be taken jointly by all the Commonwealth Governments involved. Here, it would seem that the Commonwealth Educational

* The Universities: a Royal Commission? By G. C. Moodie. (Fabian Research Series, No. 209.) Pp. 52. (London: Fabian Society, 1959.) 5*s*.

Conference has already established the appropriate means. Indeed, of the seven questions dealing with Commonwealth relations detailed by Mr. Moodie, at least five or six came under consideration at the Oxford Conference last July.

It is only fair to point out that Mr. Moodie recognizes that not all the questions he lists need be answered by the proposed commission in detail, and that decisions on some might more appropriately be left to the University Grants Committee or the universities themselves. The terms of reference he proposes, however, are comprehensive: "to examine university education to-day, the role of the universities in modern Britain, and the ways in which the universities may best be enabled to fulfil that role in the foreseeable future; in making this examination to pay special reference to the number of students to be admitted to the universities, the internal government of the universities, the special positions within the nation of the Universities of Cambridge London and Oxford, and the proper relationship with the public as represented by the institutions of Government, and with the rest of the educational system". He recognizes that the commission would require a special trained staff and that it would also need to make extensive use of sub-committees; but it is nowhere clear why, if the University Grants Committee were suitably strengthened on similar lines, it could not undertake the task at least as effectively. In any event, questions regarding the work of the University Grants Committee itself, its relations with the Treasury and the effectiveness of its control over the universities and of public accountability generally could only be satisfactorily handled by an independent body appointed by the minister concerned. Probably, as already suggested, this aspect could well fall within the review of the nation's scientific resources to which Lord Hailsham has already addressed himself.

From what Lord Hailsham has already stated, he intends to examine other questions which Mr. Moodie lists, such as the relations between the universities and the research institutes, the best means of encouraging university research, and the increase of private benefactions, particularly from industry, and for universities other than Oxford, Cambridge and London. Besides such problems of postgraduate study and research and the general financing of the universities, the specific questions listed by Mr. Moodie fall into three groups: specific problems of university expansion, including the limits imposed by the availability of qualified staff, problems of wastage, standards of admission, awards, residence and the like; the range and variety of university institutions; and university government and autonomy. The groups are not indeed sharply separated, but by and large the essential issues are clearly enough displayed; some large fields are expressly excluded, although they could be disregarded in any survey which sought to guide long-term university policy and particularly to influence the proportion of the national resources which should be allocated to the universities.

Whatever reservations may be entertained as to the means by which such questions as Mr. Moodie propounds should be examined, little exception can be taken to the propositions on which he affirms any university policy should be based. There will undoubtedly be an increasing number of school-leavers capable of profiting from, and demanding opportunities for, further education or instruction. The country needs more trained and highly trained citizens if it is not to decline into international insignificance and internal stagnation. Within any educational system, variety is essential and uniformity strongly antipathetical to choice and experiment, without which education must suffer in the long run. However these requirements are met, it will be costly, but it will be a productive and constructive form of national expenditure.

Of the first two propositions, it might be observed that further education does not necessarily mean university or higher technological education. The question of balance is as important as that of the amount of potential talent at present missed if full use is to be made of our resources of man-power—and incidentally, if the maximum satisfaction is to be given to the student not simply in his or her years of training but also in after-life. It might be added that due attention to this aspect is likely to afford the nation the most productive return from its expenditure on education at whatever level that is fixed.

Mr. Moodie is obviously opposed to the universities continuing to determine their own policies of expansion, and is dubious as to the alternative possibility of raising university standards and making wider use of other institutions for the less gifted. He seems to lean rather to a lowering of standards to something approaching the American level, though he admits that if this were done it would be essential to develop some institutions such as Cambridge, Oxford and possibly others to provide a particularly high level of education to highly selected students. He suggests that while this policy would mean that university education would become a comparatively common experience, this would be socially beneficial; and from this point of view would presumably repay the higher investment. Restriction of university entry by raising standards would also be beneficial, as it would ensure that the universities were not the only source of social leadership, and would allow them to stand more clearly for learning and research.

It seems likely that such fundamental issues as these will receive at least preliminary attention from Lord Hailsham, and if he considers that they need closer consideration by a Royal Commission, the recommendation would come with greater weight from his new position as Minister for Science. Probably in the first instance he will recommend, however, some further strengthening of the University Grants Committee so as to enable it to give more consideration to questions of long-term policy. The questions, however, of the effectiveness of the University Grants Committee itself, of the relations between the universities and the Government, the

magnitude of the resources which should be placed at their disposal and the channels by which they should be made available are certain to be examined independently by Lord Hailsham. His comments and recommendations here will be awaited with as much eagerness and interest as his proposals regarding relations between the Minister of Education, the Chancellor of the Exchequer, the Lord Privy Seal and other departmental ministers, who are in some degree responsible by the training of scientists and technologists and the use of their services.

CURRENT RESEARCH ON REPRODUCTION AND FERTILITY

Studies on Fertility

Including papers read at the Conference of the Society for the Study of Fertility, London, 1958. Edited by Prof. R. G. Harrison. (Vol. 10 of the Proceedings of the Society for the Study of Fertility.) Pp. x+176. (Oxford : Blackwell Scientific Publications ; Springfield, Ill. : Charles C. Thomas, 1958.) 25*s*. net.

Proceedings of the Third Symposium on Reproduction and Infertility

Colorado State University, Fort Collins, Colorado. Edited by F. X. Gassner, in association with Rue Jensen and H. J. Hill. (Sponsored by the College of Veterinary Medicine and the Agricultural Experiment Station.) Pp. viii+273. (London and New York : Pergamon Press, 1958.) 42*s*. net.

Essentials of Human Reproduction

Clinical Aspects—Normal and Abnormal. Edited by Prof. Joseph Thomas Velardo. Pp. ix+270. (London and New York : Oxford University Press, 1958.) 110*s*. net.

The Endocrinology of Reproduction

Edited by Prof. Joseph Thomas Velardo. Pp. viii+340. (New York and London : Oxford University Press, 1958.) 120*s*. net.

A NOTABLE feature of the new volume of "Studies on Fertility" is the publication of a group of papers on the use of synthetic progestins which, when administered orally, suppress ovulation and thus prevent conception in women. Dr. Gregory Pincus, one of the investigators originally concerned in the development of norethisterone (norlutin), norethynodrel (enovid), and similar compounds as potential oral contraceptives, reports favourably on the outcome of trials in Puerto-Rican women, and shows that a recovery of normal ovulatory function and fertility takes place readily after the cessation of medication. He, and Dr. Jackson, both mention, however, the disturbing side-effects of the oral medication, such as nausea, vomiting, dizziness, headache and abdominal pain which, though infrequent, yet require further investigation before synthetic progestins are adopted on a large scale as a safe and reliable means for the control of human fertility. Other papers in the same volume reflect adequately the various lines of advance in research on the physiology and pathology of human and animal reproduction. They cover a multitude of problems ranging from psychiatric and surgical aspects of human infertility to successful attempts of inducing conception in the guinea pig by injecting spermatozoa into the peritoneal cavity. Contributions to the physiology of gametes include new observations on the transfer of mouse eggs and on the effects of varicocœle, cryptorchidism, and ischæmia on testicular function in mammals, as well as new findings relating to the occurrence of some remarkable species differences in sperm metabolism of the boar, ram, stallion, bull and man.

The volume is a good example of progress achieved by an interchange of ideas and observations between clinical investigators, concerned primarily with the problems of human fertility, and the laboratory workers whose efforts are concentrated in the main on the more basic problems underlying the reproductive processes in animals. Now that the recently announced *Journal of Reproduction and Fertility* is going to replace the "Studies in Fertility" as the official organ of the Society for the Study of Fertility, one hopes that its editors will be able to extend even further the range of papers, while maintaining in the new publication the standard which distinguished the parent journal.

Like "Studies in Fertility", the Proceedings of the Symposium on Reproduction and Infertility, held under the auspices of the Colorado State University, illustrates well the tendency among research workers in the field of reproductive physiology to pay increasing attention to comparative aspects, in an endeavour to link up studies on human and animal fertility. Of the twenty papers given at that symposium, more than half deal with problems in cattle, mainly with the incidence and causes of vibriosis, vaginitis, malignant tumours of genital organs, and sterility of the so-called repeat-breeder cow. Those interested in human reproductive disorders will find especially interesting and instructive the chapter by Nowakowski, on the classification of male hypogonadism, based to some extent on the chemical analysis of semen, and Reifenstein's survey of new long-acting progestins and their use in maintaining human pregnancy and preventing abortion.

Progress in research concerned specifically with human reproduction naturally forms the main theme of "Essentials of Human Reproduction", a text-book written by thirteen specialists representing various medical schools in the United States, and edited by Prof. Velardo, of the Yale University Medical School. An attractive feature of this book and its companion volume, "The Endocrinology of Reproduction", is the emphasis which the authors have placed on existing problems in the physiology of reproduction and on current research problems. Both books are, moreover, notable for the inclusion of numerous illustrations, some of them reproduced from the monumental "Ciba Medical Illustrations" of Netter, others originating from Robert Wabnitz. "Essentials of Human Reproduction" contains discussion of all the main stages of the normal reproductive process in man, and tackles in addition the various disturbances of human pregnancy, endocrine disorders of the testis and ovary, and different forms of infertility. "Endocrinology of Reproduction", written alike for research students and specialists, includes, in addition to chapters on male and female endocrinology, some information on closely related subjects such as the genetics of reproduction and the anatomy and embryology of the male and female reproductive systems. As with most text-books written by several authors, there is a certain amount of overlapping. Research students will also find that some chapters of the text-book are much easier to follow than

others, and they may have some difficulty in deciding which of the copious references should be selected for further reading. This, however, does not detract from the value of the book to the specialist, both as a concise and up-to-date survey of the subject and as a useful compilation of relevant literature. T. MANN

FORMALIZATION OF CONSTRUCTIVE CONCEPTS

Constructivity in Mathematics

Proceedings of the Colloquium held at Amsterdam, 1957. Edited by Prof. A. Heyting. (Studies in Logic and the Foundations of Mathematics.) Pp. viii+297. (Amsterdam : North-Holland Publishing Company, 1959.) 60*s*.

THIS volume consists of 25 articles by 23 authors. The central themes are intuitionism and inductive definitions. The two fundamental steps in the background are Heyting's formal systems to embody Brouwer's ideas on logic and mathematics, and a classical formalization of the concept of effectively calculable functions by general recursive functions. As a result of these steps, a more formal treatment, congenial to the logician, of intuitionism and constructive mathematics in general becomes possible. Much of the book is devoted precisely to questions arising from such a treatment. Since in classical mathematics we are concerned with not only natural numbers but also real numbers and real functions, a major problem is to extend the notion of recursive functions to those of objects of higher type, that is, to define computable or constructive functionals. Kleene's paper on countable functionals, the papers by Davis, and by Kreisel–Lacombe–Shoenfield deal with this problem. In addition, Kreisel and Kleene each gives an interpretation of intuitionistic analysis by applying constructive functionals.

Another problem is to 'reconstruct' classical mathematics by restricting arbitrary functions to constructive ones. This leads to the study of constructive, in particular, recursive, analysis and topology. The articles by Goodstein, Grzegorczyk, Lacombe and Specker are taken up by this. The three papers by Rasiowa and Sikorski are concerned with formal considerations on Heyting's calculus of intuitionistic logic.

Fitch gives a consistent formal system which contains the theory of combinators and "extended basic logic". Myhill proves theorems on "finitely representable functions" which appear to be essentially the same as the Herbrand recursive functions reported in Gödel's lectures of 1934. Hermes and Wette study certain problems about the "operative" logic and mathematics.

The remaining nine articles are of a more general or a more philosophical character. Heyting makes some familiar but pertinent remarks on intuitionism ; Beth comments on his own formal treatment of the intuitionistic logic. Kalmár argues against the hypothesis that every effectively calculable function is general recursive ; Péter disputes that every general recursive function is indeed effectively calculable. Löb tries to formalize concepts such as event, brain, subject, mental occurrence of a subject, perception, in terms of constructive ideas. Nelson considers the meaning of falsity in empirical characterizations of truth, as well as the situation that classical logic identifies certain constructively distinct entities. Lorenzen stresses the importance of inductive definitions for constructive mathematics. Mostowski discourses on various degrees of constructivism. Bernays gives a free-variable, complete formal system as a natural extension of the calculus of relations.

If we summarize the concepts and methods of foundational studies under the five headings of anthropologism (strict finitism), finitism, intuitionism, predicativism (inductivism), and platonism, the book is mainly concerned with intuitionism and a part of predicativism. This is perhaps not surprising since platonism falls outside the domain of constructivity, while the principles of anthropologism and finitism are not yet sufficiently clarified to admit a direct mathematical handling. HAO WANG

BIRDS OF PREY

Pirates and Predators

The Piratical and Predatory Habits of Birds. By Colonel R. Meinertzhagen. Pp. ix+230+44 plates. (Edinburgh and London : Oliver and Boyd, Ltd., 1959.) 70*s*. net.

THE author of this handsome volume emphasizes that it is a contribution and not a monograph. The value indeed lies in the record of original observations made in the course of long experience in Europe, Africa and Asia. Economical of words, the writer has a happy knack of conveying a vivid impression of what he has seen. His touch is less sure when he cites the records of other observers, but in any event he disclaims an exhaustive representation of the literature.

An opening chapter deals with various general points, such as the food preferences of individual members of a predatory species, the regular beats and hunting territories of some, relative size of the usual victims, methods of killing and of preparation for eating, and the reactions of other birds to predators. Throughout, particular attention is paid to the occurrence of 'piracy', that is to say one species robbing another of its prey, occasionally in some instances and regularly in others. The habits of mammals and reptiles receive frequent mention.

Group by group, Colonel Meinertzhagen then briefly reviews predatory and piratical habits among the birds which he rather whimsically classes as 'amateurs', those outside the Falconiformes and Strigiformes. Species by species he next deals with many of the 'professionals', the true diurnal and nocturnal birds of prey in which he is especially interested. This is the longest section of the book and covers examples of hunting for many types of prey—insects, molluscs, fish, reptiles, birds and mammals—some species being highly specialized in this respect and others more versatile. Flying prey may be secured by level pursuit or by 'stooping' from above : prey on the ground may be located from flight (quartering, soaring, hovering), from a perch or from the ground itself (secretary-bird) ; then there are the fishing hawks, the eaters of carrion and the partly vegetarian palm-nut vulture. One would have welcomed some sort of general review of these topics, in addition to the piecemeal presentation.

Finally, there is a chapter on what the author terms 'autolycism', that is to say, making use of other animals (including man) but not as victims. There

are the many birds which nowadays usually nest on buildings or which have developed feeding habits geared to human activities. There are various associations of birds with mammals, both wild and domesticated, exemplified by the habits of the cattle egret and the ox-peckers. There are associations between different species of birds, and breeding parasitism is just mentioned. As regards associations with reptiles, Colonel Meinertzhagen has personally witnessed both *Pluvianus* and *Hoplopterus* picking the teeth of crocodiles.

The colour plates are mostly from pictures by the late George Lodge, but one of a lammergeier from a tapestry by the author is of note; the drawings and photographs are from various sources.

LANDSBOROUGH THOMSON

KEEPING UP TO DATE WITH CHROMATOGRAPHY

A Manual of Paper Chromatography and Paper Electrophoresis

By Richard J. Block, Emmett L. Durrum and Gunter Zweig. Second edition, revised and enlarged. Pp. xi+710. (New York: Academic Press, Inc.; London: Academic Books, Ltd., 1958.) 12.80 dollars.

Papierchromatographie

Von Dr. Friedrich Cramer. Vierte, stark erweiterte Auflage. (Monographien zu "Angewandte Chemie" und "Chemie-Ingenieur-Technik", Nr. 64.) Pp. 215. (Weinheim/Bergstr.: Verlag Chemie, GMBH., 1958.) n.p.

Handbuch der Papierchromatographie

Herausgegeben von Dr. I. M. Hais und Dr. K. Macek. Band 1: Grundlagen und Technik. Pp. xxiv+860. (Jena: Gustav Fischer Verlag, 1958.) 58.40 D.M.

Chromatographic Reviews

Progress in Chromatography, Electrophoresis and Related Methods, Vol. 1. Edited by Michael Lederer. Pp. ix+276. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1959.) 45*s*.

PREVIOUS editions of two of these books are already well known. The new editions of Block, Durrum and Zweig's "Manual" and of Cramer's "Papierchromatographie" maintain the high standard of their predecessors. Both incorporate recent information and improvements in arrangement of subject-matter. Because they are about subjects that are still growing rapidly, it is not surprising that these books are somewhat larger than previous editions. Block, Durrum and Zweig's book is half as big again as their first edition of 1955, and has become rather bulky for a practical manual. Cramer's book has also put on weight but it is still slim enough to carry around the laboratory without undue strain. Cramer's exposition of paper chromatography remains less highly detailed and he deals only in outline with paper electrophoresis, whereas Block, Durrum and Zweig are faced with the problem of coping adequately with both techniques within the covers of one book. Block and Zweig's section is a detailed and up-to-date account of the paper chromatography of very many substances, amply illustrated with photographs, diagrams and tables of R_F values. The bibliography covers the literature up to the end of 1956 and occupies no less than 45 pages. New features in Durrum's section on paper electrophoresis include recent developments in recording and integrating densitometers, radioactive scanners and improvements in curtain electrophoresis and other methods. All the theory is now covered in one chapter instead of being split up as previously. There is also a very full bibliography (about 2,000 references as compared with 300 in 1955) and a useful classification of electrophoretic applications. Cramer's book covers much of the subject quite adequately for most purposes. It is also well illustrated and has many tables of R_F values. The colour reproductions are extraordinarily good. The book is attractively printed and will appeal primarily to German-speaking workers.

In 1954, under the editorship of Hais and Macek, the comprehensive Czech treatise "Papirova Chromatografie" was published. Afterwards, the editors decided to prepare a new edition of this work in two volumes, the first to cover fundamentals and techniques, the second to cover applications and bibliography. Also a German translation of this new edition was planned, and Vol. 1 in German was published in August last year. However, the original Czech edition, which I have not seen, was published only a few months ago. Comparing the first Czech with this German edition, the conversion has been accompanied by a great improvement in appearance and the quality of printing and paper is now high. There is no doubt that this massive work (mistakenly entitled "Handbuch") is the most complete exposition of paper chromatography that exists. Theoretical aspects are dealt with fully and the many methods and applications are covered with remarkable thoroughness. A number of features deserve mention. There are supplements to every chapter and all are collected together as an appendix which gives full directions for preparing location reagents, for pretreating substances before chromatography and for pretreating paper when special modifications of chromatography are required. Many of the R_F values are conveniently recorded in diagrammatic form. There are excellent surveys of methods for structural analysis of large molecules, particularly proteins. The book is published in Eastern Germany and copies may not be easily obtained in Western countries, though I have been informed that there is no such difficulty in Western Germany.

It is striking how frequently new editions of books on chromatography are appearing. Cramer's is the fourth since 1951, Block, Durrum and Zweig's is the third since 1952 if the "Manual" published that year by Block, Lestrange and Zweig is regarded as the first edition. Hais and Macek's is the second in four years. It would appear that authors of such books are obliged to think about preparing new editions as soon as they are published. Nevertheless, for many and perhaps most purposes the earlier editions are still quite adequate and indeed they are largely incorporated unchanged in the new editions. (It must also be admitted that some of the subject-matter has become dead wood, but there seems to be a reluctance to cut it out.) The admirable efforts of the authors to keep their books on the whole field of paper chromatography abreast of the times may eventually prove to be an impossible task, because the subject continues to grow at an ever-increasing rate. The alternative is to select a limited number of topics and give as much information as possible about each. This is the purpose of Lederer's "Progress

in Chromatography" and Vol. 1 is the first of a series to be published at intervals (how frequently is not stated but presumably yearly). The series will be concerned with all forms of chromatography and with related methods, including electrophoresis. For this volume some of the articles have been specially written while others are taken from the *Journal of Chromatography* and all are in English. There are nine articles each contributed by specialists. Two are concerned primarily with techniques, namely, "Chromatostrips and Chromatoplates" (Demole) and "High Voltage Electrophoresis" (Michl). Both are most useful contributions because the first describes an elegant technique which deserves wider recognition and the second deals with a method which is attracting more and more interest. The other articles are on chromatographic and electrophoretic methods applied to the following special groups: mould metabolites (Reio), strychnos and curare alkaloids (Marini-Bettolo and Casinovi), sterols and steroids (Neher)—probably the most thorough survey of this group that has been written—chloroplast pigments (Šesták), anthocyanins (Harborne), inorganic phosphorus compounds (Hettler) and isotope separation (Chemla). For anyone interested in these topics, the articles offer very detailed, well-presented and up-to-date information. If future volumes maintain as high a standard as Vol. 1, the series will undoubtedly form a most valuable contribution to the literature of chromatographic and related methods.

R. CONSDEN

NEW TECHNIQUES IN PACKAGING

Pressurized Packaging (Aerosols)

By A. Herzka and J. Pickthall. Pp. xi+411. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1958.) 63*s*.

THE pressurized pack is a method of packaging and dispensing small quantities of a large variety of materials and has developed rapidly in recent years. For products like hair lacquers it is probably ideal; for many others it is efficient and convenient, but expensive; and the relatively high cost may or may not offset its many advantages. The pressure pack of horticultural insecticide is excellent for treating a treasured plant, but for pest control on a large scale gardeners who are not millionaires are soon forced back to the old bucket of derris.

Herzka and Pickthall's book is an encyclopædia of the subject and is in fact source book, laboratory handbook, trade directory and recipe book all in one. A short general section explains the general methods of operation of two-phase and three-phase systems. Section B deals with propellants, containers, valves, methods of filling and the laboratory checking of finished packs from the points of view of safety, (in)flammability, spray size and pattern, moisture content, etc. A considerable mass of information is collected together in its numerous tables and graphs. Section C gives detailed instructions for the pressurized packaging of products ranging from foodstuffs to paint strippers, cosmetics to anæsthetics. The special precautions necessary in packaging these different commodities are described and ample recipes are given. The volume concludes with a glossary of abbreviations and technical terms, trade names of materials and suppliers, patents, particulars of industrial methylated spirits and a world list of suppliers of containers, valves, propellants, sealing equipment, laboratory apparatus, etc. There are both author and subject indexes.

This is a valuable reference work, well produced and printed. Errors are few. The illustrations are the weakest part. Some of the line drawings are confusing, with the essential parts on too small a scale for it to be clear how the mechanism operates (for example, Figures 64 and 65). Not a few of the half-tone illustrations are of poor quality and not very informative. These are, however, minor blemishes in a work that, containing much information in small compass, deserves wide circulation.

V. G. W. HARRISON

TECHNOLOGISTS AND TECHNICIANS

Sandwich Courses for Teaching Technologists and Technicians

By Dr. P. F. R. Venables. With a contribution by R. Ratcliffe. Pp. 160. (London: Max Parrish and Co., Ltd., 1959.) 18*s*. net.

DR. P. F. R. VENABLES is one of the most able practitioners and protagonists of technical education in Great Britain and his speeches and writings are treated with deserved respect. In this, his latest publication, he has collected information about the so-called 'sandwich' courses for producing technologists and technicians which have grown up in such mercurial fashion over the past few years. This information is set down with characteristic vigour and supported by views which are never lacking in candour or clarity. Whether the book will have much vogue, however, is open to question. Much of it contains information about routine administrative procedure necessary for running successful sandwich courses and will be already quite familiar to those concerned with running such courses.

Where the book is likely to make an impact is among those principals of smaller technical colleges who are determined not to be left off the 'sandwich band-wagon' and who appear to put the prestige of their colleges before the genuine interests of their students. Dr. Venables makes it very clear that many higher national diploma and college associate courses would never have come into being if regional advisory committees had carried out their commitments with courage to repel over-ambitious local projects. He also makes it clear that the time has come when colleges of advanced technology should take their rightful place alongside universities; heads of departments should become professors (has he not heard of the Royal Technical College, Glasgow, and Manchester College of Technology?), eminent industrialists and technologists should be appointed to the part-time staffs as consultant professors, and research fellowships should be readily available to those more interested in teaching than research.

Few would deny the strength of Dr. Venables's arguments and most concerned with the progress of technical education in Great Britain would support him. Perhaps his views about sandwich courses and related matters would have carried greater weight, however, if he had left the preparation of this book until sandwich courses had emerged from their swaddling clothes.

Automation and Society

Edited by Howard Boone Jacobson and Joseph S. Roucek. Pp. xiv+553. (New York: Philosophical Library, 1959.) 10 dollars.

A RESOLUTION accepted by the House of Commons on May 1 in a debate on automation requested the Government to undertake an intensive study of the probable consequences of its introduction and to invite industry to co-operate in effecting the necessary changes with a minimum of hardship to its employees. In the debate, which was concerned largely with the bearing of automation on employment, reference was freely made to the report on the technical trends of automation and their impact on management and labour issued by the Department of Scientific and Industrial Research some three years ago and to subsequent inquiries in this field which have been supported by the Department. The present book does not provide the type of report which was visualized in the debate as contributing most decisively to co-operation but the sections dealing with automation and responsibility and with automation and society could assist that task of education or increasing the public understanding upon which alone such co-operation can be firmly based. Apart from the first, in which Mr. P. T. Veillette outlines very clearly the development of the concept of automation, the sections of this book are the composite work of some thirty authors, that on automation applied dealing specifically with its application in some fourteen fields in the United States. The other two sections are of greater interest to British readers, but on the whole they scarcely bring out the social implications any more clearly than was done in 1950 by the Department of Scientific and Industrial Research's report. The section on automation and society includes a useful dictionary of automation, but the 37 short case-histories of automation in the United States of America and Canada compare poorly with the three case-studies in automation published by Political and Economic Planning in 1957. It is difficult to believe that the book could not have been considerably compressed with great advantage, and the inclusion of brief biographies of contributors does not compensate for the omission of an index or for the scattering of selected bibliographies, some annotated, at the ends of chapters.

R. Brightman

Metal Fatigue

Edited by Dr. J. A. Pope. Pp. xiv+381. (London: Chapman and Hall, Ltd., 1959.) 70*s*. net.

READERS of Nevile Shute will probably be surprised to realize that the unreceptive attitude of engineering designers to Mr. Honey's ideas on fatigue was until fairly recently an authentic picture of the state of affairs in the engineering industry. Several unfortunate aircraft crashes, which were proved to be initiated by fatigue failure, have, however, been in part responsible for a change of heart, and the more forward-looking engineers are now very much exercised by the problem of making allowance for the effects of vibrational stress on their structures. Their task of producing a safe but economical design is not easy; the amount of reliable data which are available is at present inadequate and, since several uncontrolled variables affect the fatigue life, a statistical approach is essential. The volume under review should be a real help; it reproduces twenty lectures given in the Engineering Department at the University of Nottingham during a Summer School on the Fatigue of Metals. The professed aim of the editor is "to present to practising engineers the known facts about metal fatigue and its effects on design". This is therefore a book primarily for engineers, although much of it will be read with profit and fascination by metallurgists and others interested in the wayward behaviour of stressed solids.

The work is divided into three main sections: the basic phenomena and, so far as it exists, their explanation; fatigue properties of engineering materials and components; and fatigue testing of engineering components. The fifteen contributors have produced a readable, clearly presented and useful picture of the state of knowledge in 1955. The engineering designer will draw some comfort from a perusal of the book, particularly from the realization that the problems of fatigue, as they affect him, are being subjected to competent and energetic scrutiny. He will see that already in some directions solid progress has been made, notably in our knowledge of the true stress intensification at cracks and notches, but he may wonder what further developments have taken place in the four years between the compilation of the material for the lectures and the appearance of this book.

K. M. Entwistle

Principles of Modern Physics

By Prof. Robert B. Leighton. (International Series in Pure and Applied Physics.) Pp. xii+795. (London: McGraw-Hill Publishing Company, Ltd., 1959.) 97*s*.

THIS addition to the present series is most welcome. The author has tried to write a book suitable for students of physics in their final year and for specialists in other subjects who require a broad general background. It caters very well for the former group. By assuming a considerable background of mathematics and classical physics a great deal of tedious digression, common in other books, is avoided. The special theory of relativity, once relegated to an appendix, has, at last, achieved its rightful place—the first chapter; atomic and molecular spectroscopy, so often the major topics, are confined to about seventy pages. More than one-third of the book is devoted to nuclear physics. The book is very comprehensive and up to date. It includes such topics as parity, band theory of solids and X-ray diffraction. Work published as recently as 1958 is referred to. Occasionally, the material becomes too condensed for easy reading, but frequently the exercises are very well designed to supplement the text. Other exercises vary from simple logical extensions of the preceding sections to difficult problems which only a very good student could be expected to solve without assistance. Rationalized M.K.S.A. units are used throughout the book, although some of the formulæ are also given in what must still be regarded as their more familiar form, and there is a useful appendix of atomic constants in M.K.S.A. units.

E. Stanley

Directory of Nuclear Reactors

Vol. 1: Power Reactors. Pp. ix+214. (Vienna: International Atomic Energy Agency, 1959.) 73.50 schillings; 21*s*.; 3.50 dollars.

THIS directory makes available the important details of power-reactor projects, throughout the world, which will be producing electric power by the end of 1962; reactors with an electric power output

of less than 2 MW. are not included. Information is presented for each reactor under the following headings: general, reactor physics, core, fuel element, heat transfer, control, reactor vessel, fluid flow, shielding, containment, turbo-generator, cost and staff. In almost every case diagrams are also provided of the fuel element, two sections of the reactor, and the fluid-flow sheet for the system. The information is as up to date as can be hoped for; in every case, except one, it is dated between December 1958 and April 1959. Useful conversion charts and space for notes are also provided. The directory is comparable with a similar compilation published a month earlier by the American Society of Mechanical Engineers. It is better than the American publication in that the information on any particular reactor is fuller and more reactors are covered; for example, the Russian reactors are included.

The book is very good value for its price, but a more substantial backing would be an improvement as the present one is very liable to damage. A minor point is that a conversion scale from mills to pence would also be a help. The few mistakes noted were all trivial. In general, such a directory is required, the International Atomic Energy Agency appears to be the best organization to carry out such work, and the first volume is a good start. A. J. SALMON

The Birds

By Oskar and Katharina Heinroth. English edition prepared by Michael Cullen. Pp. 175. (London: Faber and Faber, Ltd., 1959.) 18*s*. net.

ORNITHOLOGY provides many fascinating problems, such as how birds mate, migrate, eat, sleep, and so on, which are a source of interest to an increasing number of people. Lack of easily available and authoritative information has given rise to a number of popular misconceptions. Mr. Cullen states that Oskar Heinroth knew this because of his contact with people at the Berlin Zoo, where he was curator for many years. That is the reason given for the book which Heinroth published in 1938 and which was revised by his wife in 1954 after his death in 1945. It is said that in this English edition certain parts of the text have been brought up to date.

Though mainly and intentionally limited to certain aspects of bird biology in which Heinroth was particularly interested, for example, the development of nestlings, a wide range of information is provided which will satisfy the curiosity of many who want to know more about birds than merely their names. There is ample scope for works of this kind, which are singularly lacking among the numerous bird-books. Although appreciating the value of this one and the skill of the translator, one regrets that a need should have to be satisfied in this way, for translations, inevitably, lack the freshness of original thoughts. It is a pity that the wealth of present-day photography was not used to give the illustrations a modern look.

Mineralogical Study on Clays of Japan

By Dr. Toshio Sudo. Pp. vii+328. (Tokyo: Maruzen Co., Ltd., 1959.) 2,000 yen; 10 dollars.

THOSE who have followed the recent literature on the clay minerals of Japan will be aware of the outstanding contribution made by Dr. Sudo and his collaborators. They will probably welcome, as does the reviewer, this collected account of his researches. Although much of this material has already been published in various Japanese and foreign journals, its re-publication in volume form is justified on grounds of convenience in reference.

The author has been quite uninhibited in his approach, and this book, being a report on research in progress, will inevitably be found wide open to criticism on many points. Numerous details will have to be revised later, by the author himself or other workers. But in its imaginative application of up-to-date techniques, the book is almost a model. In most chapters, the reviewer found things to set him thinking along new lines, as well as things to disagree with.

Most of the book is devoted to specific minerals and clay deposits. The first seven chapters are general in nature, and the reviewer feels less happy about them. Arguments, at times hard to follow, often seem to tail off inconclusively.

The presentation is excellent, but the book needs an alphabetical index, even though there is a very full table of contents. The language is, alas, only adequate at best, and at worst verges on unintelligibility. The number of printing errors is more than would be tolerable in an English or American book. We cannot expect authors to be expert linguists. Surely the blame rests with the publishing house, which is one of international repute. If they publish in English, they should ensure that adequate linguistic assistance is available. D. M. C. MACEWAN

Progress in Organic Chemistry

Vol. 4. Edited by Dr. J. W. Cook. (Progress Series.) Pp. ix+256. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1958.) 50*s*.; 8.80 dollars.

THE choice of subjects for this volume is significant in that it reflects the present dominating position of natural products in organic chemistry. Also symptomatic of current thinking is the inclusion of biogenetic considerations in both a predictive and interpretative role. Of the four chapters devoted to natural products the most extensive is a very fully documented account by A. H. Cook and G. Harris on the degradation and synthesis of peptides and proteins. F. D. Gunstone reviews naturally occurring unsaturated fatty acids. Notable here are the stereospecificity of recent synthetic procedures and the large emerging group of acids from compositous plants and micro-organisms, presenting a spectrum of ethyleniclay, acetylenic and allenic unsaturation.

2-Acylcyclohexane-1: 3-diones from sources as diverse as the male fern, hop resins and certain essential oils are discussed by C. H. Hassall in a chapter which also includes the widespread lichen constituent usnic acid, the recent synthesis of which underlies the probable role of oxidative phenolic coupling in biosynthesis. That this concept may have wider significance is apparent from recent work on the spirocoumaranones griseofulvin, geodin and erdin which, together with methylene quinones, chromenopyrones and depsidones, are included in a chapter by W. B. Whalley on oxygen heterocyclic fungal metabolites. F. G. Mann surveys recent syntheses of phosphorus, arsenic and antimony heterocycles, mostly from his own laboratory. B. and A. Pullman contribute a theoretical discussion of free valency and correlate it with a selection of physical and chemical properties in aromatic hydrocarbons. Once again the chapters of this book reflect the erudition and enthusiasm of experts discoursing within their chosen fields of interest. K. H. OVERTON

RECENT WORK ON THE ELECTRON MICROSCOPY OF VIRUSES*

By D. KENNETH M. SMITH, C.B.E., F.R.S.
Agricultural Research Council Virus Research Unit, Cambridge

THE conception of a virus as a mysterious, almost supernatural, entity dating from Mayer's studies on tobacco mosaic virus in 1886 persisted up to quite recent times, and learned discussions were held as to whether viruses were particulate or not. No one, however, suggested what they were if they were not particulate, and Beijerinck's famous conception of a "contagium vivium fluidum" was still quoted. As lately as 1932, T. M. Rivers stated with a good deal of justification that the size of no virus was accurately known.

The first indication of the shape of a virus was given by two American workers, Takahashi and Rawlins, in 1933, who observed that the sap of a tobacco plant infected with the mosaic disease showed the phenomenon of double refraction when made to flow and viewed by polarized light. This indicates that the sap contained rod-like entities, an observation amply confirmed in later years.

Apart from the so-called elementary bodies of certain animal virus diseases which came within the resolution of the optical microscope, the first actual pictures of a virus particle were made by Barnard with his ultra-violet light microscope. It was only possible, however, to resolve some of the larger animal pox viruses, and no details of the particles could be seen. It was not until the invention of the electron microscope that any conception of what a virus really looked like was obtained.

The improvements in the electron microscope and the developments in the techniques for the study of viruses during the past ten years have been very great, and it is only possible to indicate them here without any technical description.

The first virus to be observed on the electron microscope was that of tobacco mosaic, and the particles were shown to be rod-shaped, as had been predicted earlier by other methods. The first electron micrographs of this virus showed only that it was a rod-shaped particle and no other details were visible. It is by comparing these early pictures with modern electron micrographs of the same virus that some idea is obtained of the great advances which have been made in electron microscopy.

Metal Shadowing

Williams and Wyckoff[1] developed a method of shadow-casting in which metal, evaporated from an electrically heated filament in a vacuum, impinges on a specimen from a sufficient distance so that the metal atoms arrive in almost parallel straight lines. The metal, being more opaque to the electron beam, casts a shadow, thereby giving a three-dimensional effect to the objects; this gives micrographs which are a great improvement on the flat and rather characterless pictures previously obtained. Metals for shadow casting should not show a granularity which might obscure the structural detail of the object; several have been used, and they include chromium, gold and platinum alloys, and uranium.

Thin Sections

The development of the ultra-microtome for cutting ultra-thin sections has marked a great advance in electron microscopy. In the early days sections were cut at a high speed and of a wedge shape, the thin end only being examined. Nowadays, however, the technique has become almost ordinary laboratory procedure. There are various types of ultra-microtomes on the market; one or two still make use of steel knives, but the glass knife, made by fracturing a strip of plate glass, is more widely used. Recently, the diamond knife, made from a polished industrial diamond, has become popular. The advantages of this are its extreme hardness, its durability—it can be used continuously instead of only once as with the glass knife—and its suitability for cutting hard objects. Very fine results have been achieved with the diamond knife but it requires great care in its manipulation (Figs. 1 and 2).

A good operator with either glass or diamond knives should be able to cut sections of 300–400 A. in thickness.

The embedding medium is important, the most popular being a plastic made by the polymerization of butyl and methyl methacrylate[2]. Other media are 'Vestopal' polyester resin[3] and 'Araldite' epoxyresin[4]; all these require dehydration with ethanol. Just recently, however, another embedding medium, to which the name 'Aquon' is given, has been described; this medium is miscible in water and avoids the necessity of using dehydrating agents[5].

New Staining Methods

The standard method of fixing virus materials for electron microscopy is to use buffered 1 per cent osmium tetroxide, and this acts as a stain as well as a fixative. Recently, however, a number of new staining techniques have been developed; one of the most successful of these is phosphotungstic acid, and Selby[6] has used this acid at a concentration of 1 per cent adjusted to *p*H 5·4. Other useful stains are 1 per cent phosphomolybdic acid buffered at *p*H 5·4 with phthalate buffer[7], chrome gallocyanin[8], 0·1 *M* ferric chloride[9], mercuric bromophenol blue[10] and 0·5 per cent uranium acetate buffered at *p*H 6·1 with veronal acetate buffer[11]. The last-named has proved very successful with insect virus sections.

Supporting Films

For several years the standard supporting film on the grid was one of collodion or 'Formvar', and these are still used for routine work. The increasing resolution of the electron microscope and the decreasing thickness of the sections, however, led to

* Substance of a University of London Special Lecture delivered on October 29.

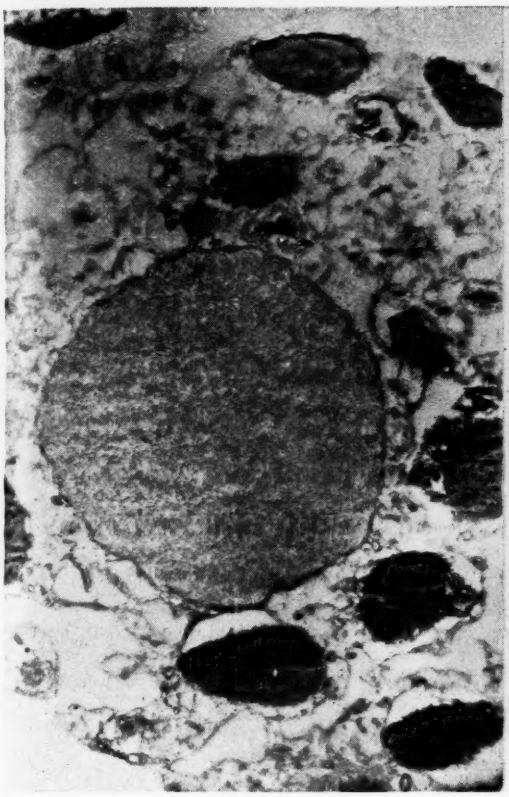

Fig. 1

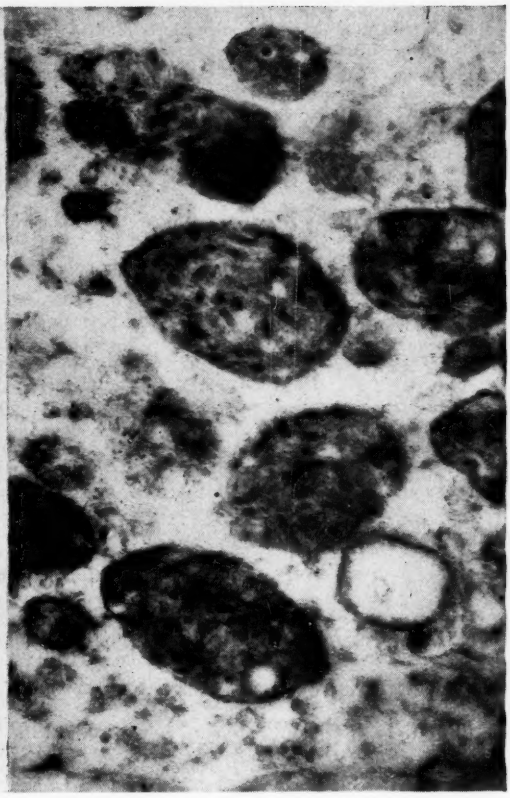

Fig. 2

Fig. 1. Ultra-thin section of a blood cell from the larva of *Tipula paludosa*, the crane fly, infected with a nuclear polyhedral disease; the virus has left the nucleus where it develops and has become occluded in the polyhydral crystals surrounding the nuclear membrane. × 10,000

Fig. 2. Similar section to Fig. 1 showing peculiar virus inclusions in the blood cell. × 49,000

difficulties and it became necessary to find another substrate. Carbon films[12] made by boiling carbon in a vacuum have proved very satisfactory; they have virtually no structure to confuse the issue at high magnifications, and they are more resistant to the electron beam and do not volatilize and foul the gun.

Carbon Replicas

Several replica techniques have been devised since the electron microscope became a laboratory tool, but except for specialized work the carbon replica has proved the most successful. This technique was first suggested by Mühlethaler, but has been brought to its present high standard by Bradley, working at the A.E.I. Research Laboratory at Aldermaston. The carbon replica technique is a single-stage process in which the suspension of material to be replicated is dried down on to a 'Formvar'-coated grid. This is coated with carbon from a source normal to the grid surface and the material removed. The source of carbon consists of two pointed carbons, spring-loaded and touching at the points. A heavy current is then passed across and a spot temperature of 2,000° C. is reached; the carbon boils and a thin coating of structureless carbon is deposited on the grid. The 'Formvar' and material are then removed by their respective solvents (Fig. 3).

This technique has been applied in various fields and a resolution of 7 A. has been obtained.

Freeze Drying

The most serious source of structural deformation in biological specimens is the force of surface tension, and attempts have been made to adapt the old-established technique of freeze drying to electron microscopy. Two methods are current, one devised by Anderson[13] and the other by Williams[14].

Anderson's technique avoids surface tension forces by drying out in liquid carbon dioxide. The specimen is fixed in osmic acid vapour, dehydrated and finally obtained in a suspension with amyl acetate. The amyl acetate is washed out with liquid carbon dioxide under 30° C. The temperature is raised through the critical point of carbon dioxide (31° C.) and pressure released. This method suffers from the fact that changes in the specimen have been brought about by the introduction of solvents.

Williams's method is more suited for general work. A suspension of the liquid is sprayed on to a carbon-coated copper disk held at the temperature of liquid nitrogen. The disk is put in a vacuum, the temperature raised to −50° C., and the ice allowed to sublime. The carbon film is removed and placed on specimen grids ready for examination.

By the application of these modern techniques to the electron microscopy of viruses, it is now possible to obtain information on the following aspects: (1) size and shape of virus particles; (2) topography of virus particles; (3) surface detail; (4) internal structure; (5) methods of replication; (6) behaviour of viruses in the cell; (7) virus crystals; (8) latent viruses.

Size and Shape

Since little internal structure is visible in whole virus particles, most of the information on their size and shape has been obtained from shadowed specimens. Variations in size and shape are considerable; as we have already seen, some of the plant viruses are rod-shaped, that of tobacco mosaic virus measures 15 mμ × 300 mμ. Other rod-shaped plant viruses include potato viruses *X* and *Y* and a number of soil-borne viruses, some of which are latent in their plant hosts.

Many plant viruses are spherical or nearly spherical, and are very small; examples are the viruses of tomato bushy stunt, Southern bean mosaic, turnip yellow mosaic, tobacco ringspot and squash mosaic, all of which range in size from 30 to 27 mμ. These very small viruses are extremely uniform in size and shape and so are easily obtained in crystalline form. While the majority of the viruses affecting animals and the higher plants are either rod-shaped or nearly spherical, a number of the bacterial viruses are tadpole- or sperm-shaped with a 'head' and a 'tail'.

Air-dried preparations of virus particles in the electron microscope are apt to show distortion and appear smaller than they actually are. Accurate estimation of size, therefore, can only be made on frozen-dried specimens.

Topography of Virus Particles

It was suggested by Crick and Watson[15] that all small viruses are built up on a framework of identical protein sub-units packed together in a regular manner. They showed that three types of symmetry are possible for spherical viruses, namely, tetrahedral, octahedral and icosahedral.

Although many of the smaller viruses present a polyhedral appearance, it requires a combination of the freeze-drying and double-shadowing techniques to give the three-dimensional shape of the particle. A good example of this is provided by the *Tipula* iridescent virus, which even in air-dried specimens presents an almost perfect hexagon or pentagon. Although this virus is rather large, it exhibits such extreme uniformity of size that it easily crystallizes.

It has been shown by Williams and Smith[16] that the particle of this virus is an icosahedron with twenty sides. A cardboard model of an icosahedron was made and shadowed by two light-sources separated 60° in azimuth and orientated so that an apex of the hexagonal contour points directly towards each light source. Under these conditions two shadows are thrown: one has five sides and is blunt-ended and the other has four sides and is sharp-ended. When a frozen-dried particle of *Tipula* iridescent virus is treated similarly, two shadows identical with those thrown by the model are produced. By means of this shadow-analysis, it is proved that the *Tipula* iridescent virus particle is an icosahedron.

Surface Detail

Until recently the amount of external structure of virus particles which could be seen on the electron microscope was somewhat disappointing. It was not possible, for example, to make out the helical structure of the tobacco mosaic virus rod as revealed by X-ray studies. The new staining methods, however, especially negative staining with phosphotungstic acid[17], have given much improved results. By this means the arrangement of the protein sub-units of the virus particle can be visualized directly on the microscope.

Particles of adenovirus, type 5, have been examined in this way. At high magnification the particle has a roughly hexagonal outline and is clearly facetted, each facet being an equilateral triangle with a side consisting of six sub-units. The presence of triangular facets immediately excludes a dodecahedral form and suggests that the particle is an icosahedron. A model of an icosahedron with a side of six sub-units was built from table-tennis balls, and the arrangements of the sub-units in the virus particle and the model are identical. Herpes virus shows a similar sub-unit structure, but the sub-units appear to be hollow and ring-shaped[18].

The same technique has been applied to one of the bacterial viruses, φ*X*174, which apparently has a nearly spherical shape without the 'head' and 'tail' of the *T*-even 'phages[19]. The micrographs of this 'phage suggest that it contains a shell of twelve 'knobs' arranged regularly as on the faces of a regular dodecahedron. This is based on the observation of one apical and five surrounding peripheral knobs with the suggestion of a second underlying tier similarly arranged.

It has been suggested by Klug, Finch and Franklin[20] from X-ray data that the particle of turnip yellow mosaic virus is a snub dodecahedron made up from sixty spherical sub-units. Electron micrographs of this very small plant virus by Brenner and Horne using negative phosphotungstic acid staining show the presence of a number of sub-units.

A characteristic of the insect viruses is the presence of membranes surrounding the virus itself and the various ancillary structures associated with them.

In the early stages of infection with one insect virus, the *Tipula* iridescent virus, there occur many empty shells, the significance of which is discussed later. By means of phosphotungstic acid staining these shells appear to consist of a double membrane, two concentric hexagons. In addition, by negative phosphotungstic acid staining, protein sub-units on the surface of the full or apparently mature virus particles become visible.

Internal Structure

Improvements in the technique of cutting ultra-thin sections not only allow sections to be cut of the virus particle itself but also of serial sections through one particle. This obviously is one way of examining the internal structure of the virus, but the difficulty here is that the electron microscope cannot differentiate between the chemical constituents of the virus. Sections do, however, reveal differences in structure and quantities of contents and, as we shall see, show up the empty virus particles.

Another method is to treat the virus particles with enzymes which dissolve away the protein covering and leave behind the nucleoprotein.

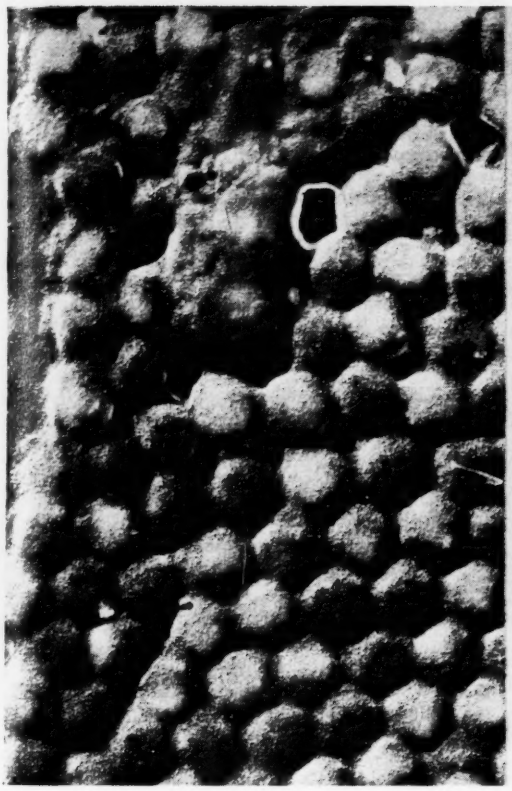

Fig. 3

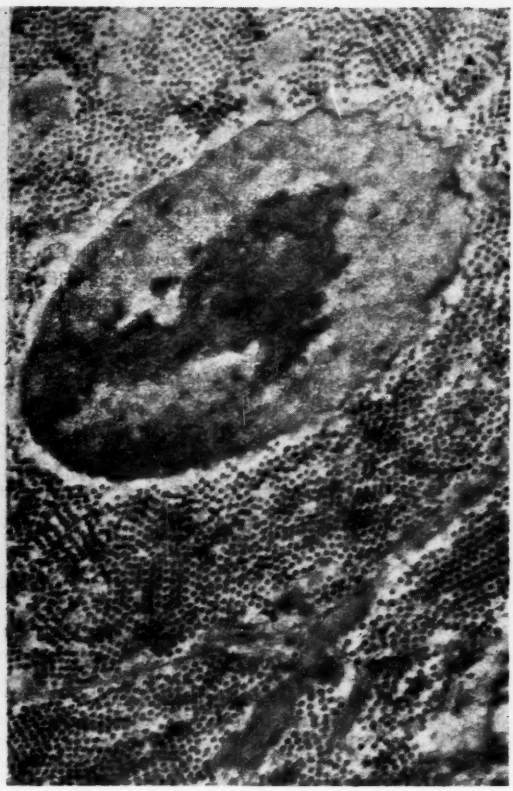

Fig. 4

Fig. 3. Carbon replica of an air-dried microcrystal of the *Tipula* iridescent virus. × 70,000

Fig. 4. Ultra-thin section of fat-body from a larva of *Tipula paludosa* infected with the iridescent virus; note how the virus avoids the nucleus and how the particles are orientated in regular array. × 10,000

Electron micrographs Figs. 1–4 by G. J. Hills

For studying the internal structure of virus particles by ultra-thin sectioning it is, of course, better to choose the larger viruses, although this technique, using a diamond knife, has been applied to tobacco mosaic virus[21].

Ultra-thin sections of vaccinia and fowl-pox viruses reveal a limiting membrane, a dense eccentrically placed body and a finely granular matrix. Sections of these viruses at a later stage seem to reveal developmental changes in which an elongated body surrounded by dense material can be seen[22].

Thin sections of the *Tipula* iridescent virus reveal a variety of different forms ranging from apparently empty shells to the mature virus with its two concentric hexagons.

The rickettsiæ, which strictly speaking should not be regarded as true viruses, are very suitable for this type of study. Thin sections of purified *Rickettsia burnetii* reveal a limiting membrane within which lie a granular region and a dense central body. The configuration of the central body suggests that it may consist of an elongated and irregularly twisted strand[23].

The method of digesting the protein covering and observing the residue by means of the electron microscope has only been applied to the larger viruses. When the influenza virus was treated on the microscope grid with various enzymes, the nucleoprotein component was left behind in the form of an irregular ring built of long threads. A similar result has been obtained with the virus of Newcastle disease of fowls, and when stained with phosphotungstic acid the ring showed a number of dark lines, possibly representing the tracks along the protein to which the long nucleic acid molecule is bound[24].

Methods of Replication

The way in which viruses reproduce themselves has always been a subject of great interest and it is only recently that definite information on this subject has been obtained. Although we know that they do not grow and divide but replicate themselves from their genetic material, it is probable that the method of replication is not the same for all viruses. The most precise information has been obtained on the multiplication of the *T*-even 'phages, and their method of replication is closely bound up with their possession of a 'head' and 'tail'. The 'phage particle attaches itself to the bacterial cell wall which it penetrates and the deoxyribonucleic acid contents of the head pass down into the bacterium[25]. What happens next has been well shown by Kellenberger *et al.*[11]. They used a 1 per cent osmic acid fixative dissolved in

acetate veronal buffer, stained their preparations with uranyl acetate and embedded for sectioning in 'Vestopal *W*'. In an infection of *Escherichia coli* by T_2 'phage, the breakdown of the bacterial nucleus is accompanied by the formation of marginal vacuoles containing deoxyribonucleic acid, from which the pool of 'phage deoxyribonucleic acid develops some minutes later. The phage deoxyribonucleic acid starts to increase 6–7 min. after infection. Then, about 9 min. after infection, the protein precursors, immature 'phages and finally, at 13 min., complete 'phage particles appear in the infected cell.

Ultra-thin sections of the fat-body of a larva of *Tipula paludosa* infected with the iridescent virus in an early stage of the disease reveal few virus particles but large numbers of empty or partially empty shells. Some of these membranes contain a few threads, others a central body which varies in size from a small granule to a darkly staining mass filling the membrane. It has been suggested that these empty membranes may represent a stage in the development of the virus; the assumption being that the membranes form first and the nucleoprotein is afterwards introduced into the empty shell. In sections fixed in formalin it is possible to see the membranes apparently being formed from the substrate.

Behaviour of Viruses in the Cell

The technique of ultra-thin sectioning has allowed the virus to be observed *in situ* in the cell, and a good deal of information on its behaviour has been obtained. Perhaps the least successful in this direction have been the studies of viruses in plant cells. This is partly due to the difficulty in distinguishing between the virus and the normal cell constituents and is particularly true of the very small plant viruses. It occasionally happens, however, that the virus may be concentrated in a small area and then becomes easily visible. In ultra-thin sections of *Datura stramonium* infected with tomato bushy stunt virus, micro-crystals of the virus may sometimes be seen.

It is much easier to observe the animal viruses in the cells, particularly when grown in tissue culture. Certain insect viruses, too, are very suitable for observation in thin sections of infected tissue.

It was shown by Morgan *et al.*[26], by means of sections of chorioallantoic membrane cells infected with herpes virus, that the virus differs slightly according to whether it is in the cytoplasm or the nucleus of the cell. The intracellular cytoplasmic particles consist of a dense central body, surrounded by a double membrane. In the nucleus, however, the particles contained the same central body but only one membrane.

The rate of development of herpes virus has been followed in cultures of HeLa cells. Samples of cells exposed to heavy infection were sectioned and examined for virus at successive time intervals after infection. No increase in infective virus was found in the cultures at 9 hr., but at 12 hr. new virus appeared in the cell fraction and particles were found in the nuclei of two of twenty-five cells. At 26 hr. after infection and later, large numbers of virus particles were present in both nucleus and cytoplasm and on the cell surface[27].

Sections of fat-body from the larvæ of the fly *Tipula paludosa* infected with the iridescent virus reveal enormous quantities of virus in the cytoplasm of the cell but never in the nucleus. The concentration of the virus is such that it forms liquid crystals in the living insect, giving rise to a characteristic blue or violet iridescence (Fig. 4).

Another virus found attacking the same fly larva causes a polyhedral disease, so-called because of the polyhedral bodies which develop in the tissues and which contain the virus. They are protein crystals, not virus crystals. In this disease the virus develops in the nucleus of the blood cells and not, like the foregoing, in the cytoplasm. The virus particles are rod-shaped and are enclosed in a membrane. After formation in the nucleus, the virus rods become occluded in the polyhedra, which lie on the outside of the nuclear membrane (Fig. 1).

Virus Crystals

Because of their small size and great uniformity, a number of viruses have been obtained in crystalline form. This is particularly true of the plant viruses, and examples are those of tomato bushy stunt, tobacco necrosis, turnip yellow mosaic, Southern bean mosaic and others. The crystals of tobacco necrosis virus are very suitable for electron microscopy because of their flat shape and resistance to the electron beam. Replicas of these crystals show clearly the orientation of the virus particles in the crystalline lattice.

After the crystallization of the plant viruses, the production of some animal viruses in the crystalline state passed without much comment. The first of these viruses to be crystallized was that of poliomyelitis and the next the *Tipula* iridescent virus. Crystalline inclusions of herpes virus and adenovirus have also been observed[22]. The crystal structure of *Tipula* iridescent virus is face-centred cubic with the particles held at a distance apart by long-range forces. These crystals show Bragg reflexions of visible light and are responsible for the blue or violet colour of infected larvæ.

We have seen already that many of the small viruses are polyhedral in shape. Electron micrographs of replicas of a plane surface cut through the interior of a single crystal of poliomyelitis virus show this very well[28].

Latent Viruses

Latent infection is an interesting phenomenon which occurs commonly. It is frequently met with in plants and even more frequently in insects, while so many new viruses have been discovered in the excretions of man and monkey that they are known as 'viruses in search of a disease'.

On the whole, attempts to visualize latent viruses *in situ* on the electron microscope have been disappointing, though rod-shaped particles have been observed in healthy plants.

It does not seem possible to stimulate a truly latent plant virus into activity; it is necessary to inoculate it into another host in order to produce a disease. This, however, does not apply to latent virus infection in insects, which can be 'triggered off' in a variety of ways. This phenomenon has been well demonstrated during cross-inoculation studies. It frequently happens that larvæ, which have been inoculated with a foreign virus, die of a virus disease. Examination, however, may show that the virus which killed the larva was not the one injected but a latent infection stimulated by the foreign virus. This phenomenon has interesting repercussions,

because it may be used to obtain new viruses from insect orders in which no virus disease has been recorded. For example, until recently no virus had ever been found in the order Neuroptera, but now two or more have been discovered in the carnivorous larvæ of *Chrysopa* and *Hemerobius*, better known as 'lacewing flies' or 'golden eyes'. Their predacious larvæ live mainly on aphids but can be induced to feed on pieces of caterpillar infected with a polyhedral virus disease. A fairly high percentage of the larvæ that do this die about ten days later from a very similar polyhedral disease (Sidor, C., unpublished work; see also ref. 29).

It has long been an axiom that insect viruses were very specific and that transmission to other species did not occur or only rarely did so. Experiments to test the truth of this were difficult, owing to the complication of latent infection referred to above. However, this difficulty has been overcome with the discovery of the *Tipula* iridescent virus; this gives the opportunity of repeating the cross-transmission studies in a way that avoids the possibility of confusing latency with transmission. It is unique in the sense that from an infected insect the virus can be isolated in the form of a brilliant iridescent pellet. No other known insect virus has this property, and so *Tipula* iridescent virus can be used as a marker and identified with certainty. With this virus it has been possible not only to infect a number of very different fly larvæ but also to transmit the virus back and forth between three different orders, the Diptera, Lepidoptera and Coleoptera[30].

[1] Williams, R. C., and Wyckoff, R. W. G., *J. App. Phys.*, **17**, 23 (1946).
[2] Newman, S. B., Borysko, E., and Swerdlow, M., *J. Res. Nat. Bur. Stand.*, **43**, 183 (1949).
[3] Ryter, A., and Kellenberger, E., *J. Ultrastruct. Res.*, **2**, 200 (1958).
[4] Glauert, A. M., and Glauert, R. H., *J. Biophys. Biochem. Cytol.*, **4**, 191 (1958).
[5] Gibbons, J. R., *Nature*, **184**, 375 (1959).
[6] Selby, C. C., *J. Biophys. Biochem. Cytol.*, **1**, 429 (1955).
[7] Smith, K. M., and Hills, G. J., *J. Mol. Biol.* (in the press).
[8] Bahr, G. F., *Exp. Cell. Res.*, **5**, 551 (1953).
[9] Bernstein, M. H., *J. Biophys. Biochem. Cytol.*, **2**, 633 (1956).
[10] Harris, P., and Mazia, D., *J. Biophys. Biochem. Cytol.*, **5**, 343 (1959).
[11] Kellenberger, E., Ryter, Antoinette, and Séchaud, J., *J. Biophys. Biochem. Cytol.*, **4**, 671 (1958).
[12] Hall, C. E., and Litt, M., *J. Biophys. Biochem. Cytol.*, **4**, 1 (1958).
[13] Anderson, T. F., *Trans. N.Y. Acad. Sci.*, **13**, 130 (1951).
[14] Williams, R. C., *Exp. Cell. Res.*, **4**, 188 (1953).
[15] Crick, F. H. C., and Watson, J. D., *Nature*, **177**, 473 (1956).
[16] Williams, R. C., and Smith, K. M., *Biochim. Biophys. Acta*, **28**, 469 (1958).
[17] Brenner, S., and Horne, R. W., *Biochim. Biophys. Acta*, **34**, 103 (1959).
[18] Horne, R. W., Brenner, S., Waterson, A. P., and Wildy, P., *J. Mol. Biol.*, **1**, 84 (1959).
[19] Hall, C. E., Maclean, Elizabeth C., and Tessman, I., *J. Mol. Biol.*, **1**, 192 (1959).
[20] Klug, A., Finch, J. T., and Franklin, R. E., *Biochim. Biophys. Acta*, **25**, 242 (1957).
[21] Fernández-Morán, H., and Schramm, G., *Z. Naturforsch.*, **13b**, 68 (1958).
[22] Morgan, C., and Rose, H. M., in "Viral and Rickettsial Infections of Man", 28 (1959).
[23] Stoker, M. G. P., Smith, K. M., and Fiset, P., *J. Gen. Microbiol.*, **15**, 632 (1956).
[24] Valentine, R. C., *New Scientist*, **6**, 567 (1959).
[25] Hershey, A. D., and Chase, M., *J. Gen. Physiol.*, **36**, 39 (1952).
[26] Morgan, C., Godman, G. C., Howe, C., and Rose, H. M., *Trans. Assoc. Amer. Physicians*, **71**, 281 (1958).
[27] Stoker, M. G. P., Smith, K. M., and Ross, R. W., *J. Gen. Microbiol.*, **19**, 244 (1958).
[28] Steere, R. L., and Schaffer, F. L., *Biochim. Biophys. Acta*, **28**, 245 (1958).
[29] Smith, K. M., Hills, G. J., and Rivers, C. F., *J. Insect. Path.* (in the press).
[30] Smith, K. M., and Rivers, C. F., *Virology*, **9**, 140 (1959).

COMPARATIVE STUDIES OF ULTRASTRUCTURE IN BIOLOGY

THE success with which the electron microscope has been used in biological research during the present decade was usefully brought to the attention of a wide variety of biologists by the Linnæan Society of London when it organized a one-day symposium entitled "Comparative Studies in Ultrastructure", held in the British Museum on April 24.

Presenting his paper, "Microsomes and Ribonucleoprotein Particles", Dr. G. E. Palade (Rockefeller Institute, New York) began by surveying the research which set the stage for one of the outstanding achievements of electron microscopy, the identification of certain small particles in the cytoplasm of cells of many different kinds as the sites at which one of the steps in protein synthesis is very probably carried out. The advances in the techniques of specimen preparation which made this possible, and had also benefited the other investigations discussed in this symposium, were in several instances due to work carried out at the Rockefeller Institute.

At the time when electron microscopy began to be applied to the problems of cell growth and the manufacture of secretory products by cells, it was believed that ribonucleic acid was intimately concerned. It was also known that the sites of this substance in the cytoplasm were basophilic, and that microsomes (one of the cell components isolated by fractionation procedures) were rich in ribonucleic acid. Dr. Keith Porter and Dr. Palade and their colleagues then discovered the submicroscopic 'endoplasmic reticulum' in the intact cell, identified it with the basophilic material seen in the light microscope and showed that the microsomes were derived from it. Palade observed that the membranes of the reticulum often, but not always, carried numerous small dense particles, about 150 A. in diameter. Using electron optical and biochemical techniques in parallel, Palade and Siekevitz demonstrated that these small particles were rich in ribonucleic acid and that the basophilia of cytoplasm and the ribonucleic acid content of microsomes were due to these ribonucleoprotein particles and not to the membranes of the reticulum. But not all the particles are attached to the membranes; many lie 'free' in the cytoplasm.

Meanwhile, it had been shown by other workers that microsome preparations could incorporate labelled amino-acids into their proteins. Palade and Siekevitz have recently studied the synthesis of chymotrypsinogen and trypsinogen and the turnover of ribonucleic acid in pancreatic cells. Various cell fractions (microsome-attached ribonucleoprotein particles, 'free' ribonucleoprotein particles, 'soluble' ribonucleic acid, etc.) have been separated and their radioactive isotope and enzyme contents measured. The results clearly implicate the ribonucleoprotein particles in enzyme manufacture and suggest that their role may be to arrange activated amino-acids

in specific sequences in the polypeptide chains of the proteins they are synthesizing.

The recent technical advances in the preparation of tissues for electron optical studies were admirably illustrated in a paper on "The Fine Structure of Vertebrate Neurons and Their Processes" by Dr. S. A. Palay (U.S. National Institutes of Health, Bethesda). He gave a detailed description of the neuron and brought out the subtle differences in structure between axons and dendrites on which depend the interpretation of the very complex pictures seen in electron micrographs of brain sections. Thus, dendrites contain large numbers of long tubules, about 200 A. in diameter, and clumps of Nissl substance, that is to say, endoplasmic reticulum bearing ribonucleoprotein particles. Axons, on the other hand, are distinguished by large numbers of neurofilaments, about 100 A. in diameter; Nissl substance is absent. These neurofilaments have approximately the same dimensions as the fibrous-protein molecules isolated by Maxwell and his colleagues from the axoplasm of the giant nerve-fibres of squid. Dr. Palay attributed the positive birefringence of axoplasm to the neurofilaments because he found that this optical property is enhanced by lipid extraction.

Among other features discussed in this paper was the structure of synapses, characterized by the increased density of the apposed cell membranes and by the presence of numerous small granules on the presynaptic side, together with mitochondria and elements of the endoplasmic reticulum.

During the past few years evidence has been brought together from several sources to show that cross-striated myofibrils are made up of a longitudinal series of alternating arrays of myosin and actin filaments, giving rise to the *A* and *I* bands; furthermore, that these arrays partly overlap in the *A* bands, and slide into or out of each other as the muscle changes its length, the protein filaments themselves staying at constant length. This evidence has led to the sliding filament hypothesis of muscular contraction, which is based on biochemical and physiological as well as structural information. The hypothesis has received strong support from the results of electron microscopy, which have revealed the spatial relationships of the two kinds of filaments and shown the existence of cross-bridges between them. This work (by H. E. Huxley) and information obtained by Hanson and Huxley by different methods (which confirm and extend the interpretation of the electron micrographs) were reviewed in a paper entitled "Comparative Studies in the Contractile Structures of Striated and Smooth Muscles", by J. Hanson, H. E. Huxley and J. Lowy (Medical Research Council, King's College and University College, London).

The sliding filament hypothesis was based on studies of vertebrate skeletal muscles, and until recently too little was known about smooth muscles, or about any other contractile system, for one to be able to say whether or not a sliding filament mechanism is a special property of striated muscles. But Hanson and Lowy have now found that in three different types of smooth muscles (the byssus retractors of the mussel *Mytilus*, the translucent part of the adductor of the oyster, and the funnel retractor and mantle muscles of the squid) the contractile apparatus consists of two kinds of filaments linked by bridges which resemble those seen in electron micrographs of cross-striated muscles. In all these smooth muscles one kind of filament is about 50 A. in diameter, like the actin filaments of striated muscles. The other kind of filament is thicker, the diameter varying from one type of muscle to another. In the case of the *Mytilus* byssus retractors, a comparison of extended and contracted muscles, cut transversely, has shown that neither the diameters of the thicker filaments nor their numbers (in unit area) change as a result of length changes in the muscle. These findings indicate that the thicker filaments are discontinuous along the fibre and do *not* contract. Such electron optical evidence (together with certain results from X-ray diffraction and light microscopy) strongly suggests that these smooth molluscan muscles contract by a sliding filament mechanism. (However, the smooth muscles of vertebrates, of which Mrs. C. Schoenberg showed some electron micrographs in the discussion following this paper, apparently possess only one kind of filament, about 50 A. in diameter, and no bridges have yet been seen.)

The rest of the paper dealt with work done by Hanson and Lowy on the structure of several molluscan muscles which show various degrees of tonicity, that is to say, the ability to maintain a steady high level of tension for prolonged periods of time. These comparative structural investigations have been carried out in parallel with experimental studies on the mechanical properties of the muscles.

It has been found that the more tonic a muscle is, the longer tension persists after stimulation has ended and the active state has decayed to zero. Thus, in the tonic anterior byssus retractor of *Mytilus*, the decay of tension can be two orders of magnitude slower than that of the active state, whereas in non-tonic muscles like the frog sartorius, or the smooth funnel retractor of *Octopus*, the time scales of the two processes are of the same order of magnitude.

Electron optical studies show that muscles such as the funnel retractors of cephalopods contain well-differentiated myofibrils and sarcoplasmic reticulum, and that their filaments are regularly packed and of relatively small diameter (about 100 A. and 50 A.). In these features such muscles resemble vertebrate skeletal muscles, the chief differences being the absence of *Z* lines and of any alignment of the two kinds of filaments into transverse arrays. At the other extreme, in a specialized tonic muscle like the anterior byssus retractor of *Mytilus*, the filaments are not grouped into fibrils, but are irregularly packed, and they vary greatly in diameter, the thicker ones (that is to say, those which are not of the 50 A. kind) ranging from about 150 A. up to more than 1000 A. In addition, such tonic muscles are distinguished by the fine structure of their thicker filaments. This structure has been found to be due to the presence of large amounts of tropomyosin. The problem now is to show if, and how, tropomyosin is involved in the mechanism responsible for the tonic specialization of smooth muscles, which contract similarly to non-tonic muscles by a sliding filament mechanism.

One of the classical problems of muscle physiology, namely, the means by which the excitatory stimulus reaches the contractile elements inside a muscle fibre, has been brought a step nearer solution by the application of electron microscopy. Several earlier light microscopists described transversely-orientated structures (as distinct from the longitudinal myofibrils and their bands) in striated muscle, and already in 1881 Retzius had suggested that these structures were involved in excitation. The greater

resolution of the electron microscope has made possible a more detailed study of these structures, which are now known as the 'sarcoplasmic reticulum'. The recent work of A. F. Huxley (Cambridge) and his colleagues goes a long way towards showing that this reticulum, or probably one particular part of it, is somehow concerned in conducting the excitatory stimulus.

In a paper entitled "Comparative Study of the Activation of Contraction in Muscle", Huxley described how isolated muscle fibres were viewed in an interference microscope or in a polarizing microscope, while the potential difference across the surface membrane was changed over a very small area by passing current through a micropipette in contact with the membrane. It was found that excitation was conducted transversely (inwards into the fibre) only when current was applied at the levels of the *Z* lines in frog fibres, and near the *A* : *I* boundaries in crab and lizard fibres. But responses were not obtained everywhere as the micropipette was moved around the perimeter of the fibre at these levels; instead it was found that the sensitivity was limited to definite small areas. Stimulation at these places resulted in local contractions which extended into the fibre for a distance several times greater than the length of the sarcomere, but did not spread longitudinally into adjacent sarcomeres. Effective stimulation in the frog fibre always resulted in an equal shortening of both halves of the *I* band, the *Z* line remaining central; but in lizard or crab fibres it was possible to cause the *Z* line to move towards one *A* band, its distance from the other *A* band usually increasing as this half of the *I* band was actually stretched. All these results were elegantly and convincingly demonstrated in ciné films.

The location of the sensitive regions near the *A* : *I* boundary in crab and lizard fibres, and the finding that only one half of the sarcomere shortens, suggest that the *Z* line cannot be the structure concerned in conducting excitation. Instead, it is probable that certain differentiated elements of the sarcoplasmic reticulum—the 'triads' first described by Porter and Palade—are involved, because as Huxley showed in electron micrographs, these 'triads' are located at the *Z* line level in frog fibres, and near the *A* : *I* boundary in lizard fibres.

As the title shows, the symposium was planned to emphasize the value of the comparative approach to biological problems. The papers on muscle illustrated two successful applications of this: the identification of structures concerned with a given function (for example, transmission of stimulation into a muscle fibre) by comparing systems where these functions are differently located; and the recognition of fundamental properties (for example, the structural features which are common to all kinds of contractile systems) by comparing muscles specialized in different ways. Similarly, a comparison of different kinds of cells, particularly mammalian, which vary in the intensity and distribution of cytoplasmic basophilia, helped to locate ribonucleic acid at the sub-microscopic level. Also the importance of getting away from stock laboratory animals and looking for other more suitable experimental material was brought out by the notable success of Dr. Palay in preparing the central nervous system for examination in the electron microscope: he chose to work on goldfish. However, the only papers which dealt with invertebrate animals were those on muscles, and it was disappointing that in none of the papers was there any mention of plant cells.

J. Lowy

NEWS and VIEWS

Thermo-Nuclear Physics at Sydney : Prof. C. N. Watson-Munro, O.B.E.

Mr. C. N. Watson-Munro has been appointed, as from January 1, 1960, to the chair of physics in the field of thermo-nuclear physics in the School of Physics, at the University of Sydney. The chair normally will be vacant on the retirement of Prof. V. A. Bailey at the end of 1960, but provision has been made for the earlier filling of the chair. Mr. Watson-Munro was born at Dunedin, New Zealand, in 1915. He graduated at Victoria University College, Wellington, with first-class honours, winning the Sir George Grey scholarship and later a Jacob Joseph scholarship for postgraduate research. In 1935 he joined the Department of Scientific and Industrial Research, New Zealand, where he carried out original work on problems of seismology and geophysics at the Dominion Physical Laboratory. In 1939 he joined a team engaged on radar development of which he became the leader. In 1941 he went to the United States and worked on radar research at the Massachusetts Institute of Technology before establishing the first New Zealand Scientific Liaison Office in Washington in 1942. Later that year he returned to New Zealand to become the director of the New Zealand Radar Development Laboratory. Early in 1944 he was specially invited to join the Atomic Energy Research Project at the Chalk River Laboratories in Canada under the direction of Sir John Cockcroft. He was largely responsible for the design and operation of the first power heavy-water pile to be built. Transferring in 1946 to the Atomic Energy Research Establishment, Harwell, he was, as principal scientific officer, in charge of the team responsible for the design, construction, and operation of Britain's first atomic pile, *Gleep*, a low-power graphite pile that was constructed in fifteen months and has proved very successful. He returned to New Zealand in 1948 to take charge of the Department of Scientific and Industrial Research. Accepting the chair of physics at Victoria University, Wellington, in 1951, Prof. Watson-Munro became dean of the science faculty of that University in 1954. In 1955 he was appointed chief scientist of the Australian Atomic Energy Commission responsible for the Lucas Heights Research Establishment which was constructed under his supervision.

Theoretical Physics at Sydney : Prof. S. T. Butler

Dr. S. T. Butler has been appointed, as from November 2, 1959, to the chair of physics in the field of theoretical physics in the School of Physics, at the University of Sydney. This is a newly created chair.

Dr. Butler first studied at the University of Adelaide and graduated (B.Sc.) in 1945 with credits in all subjects (physics, pure mathematics and applied mathematics). He obtained his first-class honours degree at Adelaide in 1946, and his M.Sc. in 1947. This latter was for theoretical work, in collaboration with the Physiology Department at the University of Adelaide, and involved an analysis of the transient responses of electronic equipment being employed in physiological and biological research. In 1948 Dr. Butler obtained an Australian National University scholarship, and proceeded to the Department of Mathematical Physics at the University of Birmingham, England, to work under the supervision of Prof. R. E. Peierls. He remained there until September 1951, obtaining his Ph.D. from the University of Birmingham in June 1951. In September 1951, Dr. Butler took up a position in the Laboratory of Nuclear Studies at Cornell University, where he remained for two years. He then returned to Australia first to spend ten months at the Australian National University at Canberra, Australia, and then to take a readership at the University of Sydney. Since leaving Birmingham in 1951, Dr. Butler has made research contributions in several fields, including the low-temperature problems of liquid helium and superconductivity, and in the field of meson and strange particle physics. Most of his work, however, has been in the field of nuclear reactions, not only furthering his original work on stripping reactions, but also as one of the originators of the general field of direct nuclear reactions.

Scientific Adviser to the U.K. High Commissioner in India: Dr. H. R. Ambler, O.B.E.

Dr. H. R. Ambler has been appointed to the newly created post of scientific adviser to the U.K. High Commissioner in New Delhi. In addition to advising the High Commissioner on scientific matters, he will be responsible for furthering the exchange of information between British and Indian scientists. Dr. Ambler, who is fifty-eight, will hold the rank of senior principal scientific officer in the Department of Scientific and Industrial Research. He joined the Government service shortly after he graduated with first-class honours in chemistry at the University of London in 1920. Since then he has held a variety of posts at home and abroad, including seven years in India. At present, Dr. Ambler is assistant director of the Chemical Inspectorate of the Ministry of Supply at Woolwich. Dr. Ambler, whose appointment is for three years in the first instance, is taking up his new duties at the end of the year.

Explosives Research and Development at the Ministry of Aviation: Dr. C. M. Johnson, C.B.E.

The Ministry of Aviation announces that Dr. C. M. Johnson has been appointed director of the Explosives Research and Development Establishment at Waltham Abbey. Dr. Johnson was born in 1904 and gained his Ph.D. at the University of London in 1927. For the next eleven years he was a lecturer in chemistry at the University of California and the University of Bristol. In 1938 he joined the Shell Refining and Marketing Co. as research manager, moving in 1948 to the Explosives Research and Development Establishment. Since 1954 he has been director of the Materials and Explosives Research and Development Branch.

Deputy Director of Air Armament Research: Mr. W. H. Hill

Mr. W. H. Hill has been appointed deputy director of air armament research. He was born in 1915 at Warwick, and gained his degree at the University of Cambridge. During 1937–41 he was an assistant lecturer in applied mathematics at the University College of the South West, Exeter. In 1941 he joined the Royal Electrical and Mechanical Engineers, and was posted to work with the Ministry of Supply on radar and signals research. From 1946 until 1951 he worked at the Army Operational Research Group, where he became general scientific co-ordinator. In 1951 he joined the Department of the Scientific Advisor to the Army Council. In 1952 he was appointed to the Royal Aircraft Establishment, working on guided weapons, and in 1954 he transferred to the Armament Research and Development Establishment as superintendent of the Weapons Assessment Department.

History of Medicine and Pharmacy

A Faculty of the History of Medicine and Pharmacy has been founded under the auspices of the Worshipful Society of Apothecaries of London (which received its Charter from King James I in 1617). Its aim is to foster and extend more general interest in medical history, and to attract the co-operation of general historians, so that work in this field may be co-ordinated with wider historical studies. The Society will provide in its ancient hall a central meeting point for historians, and medical and pharmaceutical historical societies. In addition to the Gideon de Laune Oration, which will next be delivered by Sir John Charles in April 1960, it has an endowment for two additional lectures which may be delivered in London or elsewhere. A research fellowship, generously endowed by the Wellcome Trustees, is held at present by Mr. R. S. Roberts, a professional historian, who is working upon the early organization of the drug trade from the seventeenth century. It is proposed to hold occasional symposia, discussions, congresses, and joint meetings with other bodies; invitations to visit sites of medical historical interest have already been received. Announcements and reports of meetings will appear in the official organ of the Faculty, *Medical History*, which is published quarterly. Ordinary membership of the Faculty is by election. Applications for affiliation from student historical, medical or pharmaceutical societies or similar bodies, which should nominate a representative, can also be accepted. The inaugural lecture will be given in the Hall of the Society on December 2 by Canon Charles E. Raven, on "Medicine—Mother of the Sciences". Further information can be obtained from the honorary secretary, Dr. F. N. L. Poynter, Wellcome Historical Medical Library, Euston Road, London, N.W.1.

Distribution of British Books and Films Overseas

In reply to a question in the House of Commons on November 9, the Chancellor of the Duchy of Lancaster, Dr. Charles Hill, said that negotiations had begun with several countries with the aim of agreeing upon schemes broadly on the lines of the former British book export schemes. Mr. A. L. P. Norrington had accepted the chairmanship of the Advisory Committee on the Selection of Low-Priced Books for Overseas, and the necessary administrative arrangements were being made so that negotiations

on contracts could begin as soon as titles had been selected. A Publishing Trade Consultative Panel had been set up after consultation with the trade organizations concerned. The British Council was steadily expanding its own library services and co-operating with Colonial Governments in improving their services and there had been consultation with trade organizations in the periodical field on measures to assist circulation of newspapers and periodicals in some of the more difficult markets overseas. Consultation was also taking place with the publishing trade in Great Britain and other countries with the view of encouraging more effective wholesaling and retailing of books in the African countries in particular. Answering a further question, about documentary films, Dr. Hill said that in 1958–59 the Central Office of Information commissioned twenty-eight documentary films and acquired 118, all for overseas distribution, and in 1959–60 it was expected that about 120 would be acquired. More than a hundred British films had been entered in 1959 for showing at international festivals and twenty-one awards or special mentions had been gained.

National Lending Library for Science and Technology: Consultative Committee

A CONSULTATIVE Committee has been appointed to advise the Department of Scientific and Industrial Research on the services of the National Lending Library for Science and Technology. Literature is now being collected for the Library by the Lending Library Unit of the Department at 20 Chester Terrace, Regent's Park, London, N.W.1. The National Lending Library for Science and Technology will be located near Boston Spa, Yorkshire, and its transfer will take place in the first half of 1961. Its collection of scientific literature, which will become the largest in the United Kingdom, will be available to any organizations through loan and photocopying services.

The Committee is as follows: Sir Lindor Brown, biological secretary of the Royal Society (*chairman*); Prof. J. D. Bernal, professor of physics, Birkbeck College, London; Mr. D. I. Colley, librarian, Manchester Public Library; Dr. B. M. Crowther, editor of *Science Abstracts*; Mr. G. H. Davison, secretary of the United Steel Companies, Ltd., Research Department; Dr. R. E. Fairbairn, head of the Library Department, I.C.I. Dyestuffs Division, and chairman of the Council of Aslib; Mr. R. M. Fishenden, United Kingdom Atomic Energy Research Establishment, Harwell; Prof. G. Gee, Sir Samuel Hall professor of chemistry, University of Manchester; Mr. C. W. Hanson, research officer, Aslib; Mr. L. J. H. Haylor, Ministry of Supply; Mr. K. W. Humphreys, librarian, University of Birmingham, and secretary of the Standing Conference of National and University Libraries; Mr. D. D. Morgan, information manager, P.E.R.A.; Prof. W. J. T. Morgan, Lister Institute of Preventive Medicine, London; Mr. H. T. Pledge, keeper, Science Museum; Dr. M. R. Pollock, Medical Research Council; Mr. A. C. Townsend, library of the British Museum (Natural History). The secretary of the Committee is Miss R. M. Bunn.

Dounreay Fast Breeder Reactor Critical

THE Fast Breeder Reactor at Dounreay went critical on November 14. The reactor will go through a period of low-power operation for some months. This may be followed by a shut-down for any modifications found necessary, and the reactor will then be worked up to power in stages. Construction work on this reactor started in May 1955 and it was scheduled to start operating last year. Because of the redeployment of staff due to the Windscale pile incident and to certain design modifications, start-up has been delayed. The reactor consists of a central core which is a hexagonal prism approximately 21 in. high and 21 in. across the flats. This is made up of about 360 enriched uranium rods in niobium cans, and is surrounded by a 'blanket' of 2,000 rods of natural uranium which under irradiation produces plutonium. Plutonium can be used in the core to produce more heat and more plutonium, and experiments have shown that the amount of fissile material produced in the core and the blanket during operation will exceed the amount burned up in the core, and the reactor is thus a breeder. In this type of reactor the neutrons are not deliberately slowed down as in a thermal reactor, that is, no 'moderator' is used and the neutrons are said to be 'fast'. Heat is removed from the core and blanket by a liquid sodium–potassium alloy, which is circulated by electromagnetic pumps through the vessel and through heat exchangers. The reactor with its primary heat exchanger circuits is installed in a concrete vault within the 135-ft. diameter steel sphere which dominates the Dounreay site. In the early stages the Dounreay reactor will be operated to provide information on the physics and control characteristics of this type of system, and this will be followed by a stage of intensive development and testing of different types of fuel elements.

South African Council for Scientific and Industrial Research

THE thirteenth annual report of the South African Council for Scientific and Industrial Research covers the year ended March 31, 1958, and includes lists of research bursaries and assistantships awarded during the year as well as a list of publications (Pp. xvii+226+xix–lix. Pretoria: South African Council for Scientific and Industrial Research, 1958). Concern is expressed as to the shortage of scientists and particularly at the loss overseas of promising South African scientists. Much interest is being taken in the work on the demineralization of water by electrodialysis, and the plant designed by the Council and being erected at Welkom has a capacity of 2·5 million gallons a day. River surveys involved a comprehensive programme of chemical, biological, bacteriological tests and flow data, as well as laboratory studies on the chemical and biological reactions in water and river mud. A Wine Research Unit was established at Stellenbosch, where it is hoped that investigations of the causes of biotic turbidity will offer a solution of a serious problem in South African wines. Investigations on the chemical mechanism of carcinogenesis continued. Initial difficulties with the cyclotron at the National Physical Laboratory were overcome during the year and improvements in several of the basic components increased the stability of operation. The isotope separator was assembled in December 1957, and the electro-mechanical recording systems in South African Courts established by the Acoustics Division were further extended. Under a recently established Foundry Sands fellowship the properties of available sands are being studied and their suitability for casting determined. At the National Mechanical Engineering Research Institute

work was carried out on the phenomenon of rock-bursts with a view of devising methods for their prevention or at least of decreasing their incidence and severity. The Institute is also studying erosion, the silting of rivers and harbours, coal-burning gas turbines and solar operated refrigeration systems.

In telecommunications a radio tracking system was improvised which enabled the orbit of the first Russian Earth satellite to be determined roughly within a few days and an accurate determination of the velocity of radio waves was made by means of extensive measurements at the Ridgeway base and surrounding triangulation to provide a value which can be used confidently in work with 'tellurometers'. Work was carried out on the shear strength of non-saturated soils under different moisture conditions and the stresses set up in various layers of road foundations by compaction operations or surface loads were closely observed. Experimental studies on the relations between malnutrition and the mental development of young children showed that 38 per cent of a group of Bantu children suffering from kwashiorkor had abnormal electroencephalograms compared with only 3 per cent of normal children, and another study showed that rats bred from protein-deficient mothers and themselves reared on protein-deficient diets were inferior in learning ability to those bred and fed normally. In medical research particular attention is being given to the relation between diet and diseases of the arteries.

Waterways and Ionizing Radiations

Circular No. 57/59 from the Ministry of Housing and Local Government to local authorities, joint water boards, joint sewage boards, river boards, statutory water companies in England and Wales tells them from which departments of the Government they should seek advice when in doubt about local problems concerning hazards from ionizing radiations. As a potted "Inquire Within on Everything" in this field it is extremely informative and helpful. As an explanation of the scientific problems involved it is slipshod, since the construction of the sentences is tortuous, 'radioactivity' is confused with 'radiation' and there is at least one mis-statement attributed to the Medical Research Council on the contribution by diagnostic X-rays to the dose of radiation received by the general population.

Hatfield Memorial Lecture

Academician G. V. Kurdjumov, director of the Department of Metallography and Metal Physics at the Central Research Institute for Ferrous Metallurgy, Moscow, has accepted an invitation from the Council of the University of Sheffield to deliver the twelfth Hatfield Memorial Lecture, in the Firth Hall of the University on November 25. His subject will be "The Phenomena occurring in Steels during Quenching and Tempering". The Hatfield Lectures, which are given annually in either London or Sheffield, were founded in memory of the late Dr. W. H. Hatfield, and are always devoted to a subject in which Dr. Hatfield was interested. The Hatfield Memorial Lecture Fund is administered by the University of Sheffield as trustees, and arrangements are in the hands of a Lecture Committee, composed of representatives of the University, the Royal Society and the Iron and Steel Institute. Academician Kurdjumov is also to lecture to the Iron and Steel Institute during the autumn general meeting in London. He will be speaking at the Hoare Memorial Hall, Church House, Great Smith Street, S.W.1, on December 2, and on this occasion his subject will be "Martensitic Transformations in Metals and Alloys".

International Federation of Automatic Control

Following the first General Assembly in Chicago of the International Federation of Automatic Control, it is announced that the Executive Council now consists of the following members: *President*, A. M. Letov (U.S.S.R.); *Past President*, H. Chestnut (U.S.A.); *First Vice-President*, Ed. Gerecke (Switzerland); *Second Vice-President*, O. Benedikt (Hungary); *Treasurer*, M. Cuénod (Switzerland); *Ordinary Members*, J. G. Balchen (Norway), J. F. Coales (Great Britain), G. Evangelisti (Italy), K. Kaneshige (Japan), P. J. Nowacki (Poland), Z. Trnka (Czechoslovakia). D. P. Eckman (U.S.A.) will remain chairman of the Advisory Committee of which J. Loeb (France) was appointed vice-chairman.

The Vernon Prize

The late Dr. H. M. Vernon made a bequest to the National Institute of Industrial Psychology expressing the wish that the Institute should award a prize every second year to the investigator who, being a British subject and under the age of forty-five years, has in the opinion of the council of the Institute done the most valuable research work in the service of industrial psychology or industrial physiology. The fourth award will be made in 1960; it will consist of approximately £40 in cash and a silver medal. Recommendations and direct applications must be received by February 29, 1960. Further information can be obtained from the Secretary, National Institute of Industrial Psychology, 14 Welbeck Street, London, W.1.

Royal Agricultural Society of England Research Medal

The Royal Agricultural Society of England offers annually a Medal together with an award of 100 guineas for research work of outstanding merit carried out in the United Kingdom which has proved, or is likely to prove, of benefit to agriculture. Recommendations for this award can be made by the heads of university departments, research stations and institutes and other research organizations. No direct application by candidates will be considered. Recommendations for the next award must be submitted to the Secretary, Royal Agricultural Society of England, 35 Belgrave Square, London, S.W.1, not later than January 30, 1960, and from whom further information can be obtained.

Course on Digital Computers

The British Council is arranging a course on digital computers in London during February 7–20 for industrial and research scientists from overseas. The course will concentrate on recent improvements in computer design and programming techniques, and will be closely linked with practical work in industry. Many firms have already offered to show computers being made or actually used for commercial and industrial purposes. Course members will also be able to discuss problems with workers on the job. The course will be directed by Dr. Andrew Booth, head of the Department of Numerical Automation at Birkbeck College. Visits have been arranged to see computing equipment at the National

Physical Laboratory (*Deuce* and *Ace*), Ferranti (*Pegasus* and *Sirius*), Elliot Brothers, Cambridge University Mathematical Laboratory (*Edsac II*), E.M.I. Electronics (*Emidec*), London University Computational Laboratory (*Mercury*), Creeds (project high-speed punching), Solatron (*Era*), Leo Computers and International Computers and Tabulators. The fee for the course is £47 and accommodation will be in London hotels.

Radioisotopes in Agricultural Research

A REGIONAL training course on the use of radioisotopes in agricultural research, organized jointly by the Ministry of Food and Agriculture, Government of India, Food and Agriculture Organization, International Atomic Energy Agency and Unesco South Asia Science Co-operation Office, will be held at the Indian Agricultural Research Institute, New Delhi, during January 20–February 17, 1960. The course will deal with the role of radiation in cytology and genetics, radioisotope techniques as applied to problems in soil fertility, fertilizer application and plant biochemistry and radiation as a protective agent. Unesco has extended invitations to the Governments of Burma, Ceylon, India, Pakistan and Thailand. A maximum of twenty-five participants will be admitted to the course. Further information can be obtained from the Unesco South Asia Science Co-operation Office, 21 Curzon Road, New Delhi, India.

The Night Sky in December

FULL moon occurs on Dec. 15d. 04h. 49m. U.T. and new moon on Dec. 29d. 19h. 09m. The following conjunctions with the Moon take place: Dec. 2d. 06h., Saturn 4° S.; Dec. 26d. 19h., Venus 2° S. In addition to these conjunctions with the Moon, Mars is in conjunction with Antares on Dec. 15d. 16h., Mars being 4·4° N., Mercury with Antares on Dec. 20d. 08h., Mercury being 6·0° N., Mercury with Mars on Dec. 25d. 13h., Mercury being 1·0° N., Mercury with Jupiter on Dec. 27d. 01h., Mercury being 0·0° S., and Mars with Jupiter on Dec. 29d. 01h., Mars being 0·8° S. Mercury will be visible as a morning star during the first three weeks of the month, rising almost two hours before the Sun, but conditions are not particularly favourable. Venus is a morning star, rising at 3h. 25m., 4h. 00m. and 4h. 40m. on December 1, 15 and 31, respectively; its stellar magnitude is −3·8. Its distance increases during the month from 77 to 98 million miles and the visible portion of the apparent disk increases from 0·598 to 0·714. Mars, Jupiter and Saturn are too close to the Sun for observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Dec. 13d. 22h. 10·3m., 75 Tau. (*D*); Dec. 20d. 6h. 09·4m., o Leo. (*D*); Dec. 20d. 7h. 12·6m., o Leo. (*R*); Dec. 27d. 6h. 34·1m., θ Lib. (*R*); *D* and *R* refer to disappearance and reappearance, respectively. The Geminid meteors are active during December 9–14 and the Ursids during December 20–22, but in neither case are conditions favourable for observation. The winter solstice occurs on Dec. 22d. 15h.

University News: London

DR. G. MORTON, lecturer at the London School of Economics and Political Science, has been appointed to the University readership in operational research tenable at that School.

Titles have been conferred as follow: professor of embryology on Mr. Michael Abercrombie, reader in chemistry on Dr. D. J. Millen, reader in botany on Mr. P. J. Syrett, in respect of their posts at University College.

Sheffield

THE following appointments to lectureships have been announced: E. Litton (civil engineering); L. G. M. Sparling (mechanical engineering).

Announcements

THE firm of Blackie and Son, Ltd., has celebrated its 150th year of publishing. Until very recently, all the scientific and technical publishing was conducted from Glasgow, but now a publishing department has been set up at the firm's London office to handle this type of book, and Mr. Christopher T. Rivington has been appointed London scientific editor.

MR. A. S. D. BARRATT, technical director of Edwards High Vacuum, Ltd., since 1945, is resigning from the board of the Company at the end of the year to become an independent consultant. His long association with the Company will, however, be maintained by his appointment as technical consultant to the Company, thus providing a liaison between the large-scale developments of vacuum technology now taking place and the long-term developments of the Edwards international group of companies.

THE Iron and Steel Institute is devoting the main sessions at its autumn general meeting during December 2–3 to the forging and extrusion of steel. The meeting is being held in the Great Hall, Caxton Hall, London. Further information can be obtained from H. Cleere, Iron and Steel Institute, 4 Grosvenor Gardens, S.W.1.

A SYMPOSIUM on the Exploitation of Natural Animal Populations, sponsored by the British Ecological Society, will be held at the Durham Colleges, University of Durham, during March 28–31. Full details can be obtained from E. D. Le Cren, The Ferry House, Ambleside, Westmorland.

THE University of Texas M.D. Anderson Hospital and Tumour Institute announces the fourteenth Annual Symposium on Fundamental Cancer Research, "Cell Physiology of Neoplasia", to be held during February 25–27. Further information can be obtained from the Editorial Office, The University of Texas M.D. Anderson Hospital, Texas Medical Center, Houston 25, Texas.

THE Department of Civil Engineering and the Department of Extra-Mural Studies in the University of Birmingham have announced a course on River Pollution Sewage Disposal and Trade Waste Treatment, to be held at the University of Birmingham during January 4–8, 1960, and continued during April 4–8, 1960. The course is arranged in co-operation with the Institute of Sewage Purification. Further information can be obtained from Mr. R. F. Wills, Civil Engineering Department, The University, Edgbaston, Birmingham 15.

FURTHER to the announcement in the September 19 issue of *Nature* regarding the availability of the "Great Soviet Encyclopædia" in Britain, a complete translation into English of the fiftieth volume of the second edition will be published by Pergamon Press, Ltd., early in 1960 under the title of "U.S.S.R. Information". This is an authorized translation, produced with the full co-operation of the editor-in-chief of the "Great Soviet Encyclopædia".

CHEMISTRY IN THE EIGHTEENTH CENTURY

An international meeting was convened in Paris during September 11–13 by the Comité National Francais d'Histoire et de Philosophie des Sciences in the name of its president, Prince Louis de Broglie. It was timed to follow on the ninth International Congress on the History of Science, held this summer in Spain; and some of those attending did in fact reach Paris via Barcelona. The invited contributors were asked to review topics such as: phlogiston; the discovery of the gases; the preparation of non-gaseous chemicals; the reformation in chemistry; affinities; chemical biographies and works; chemistry and eighteenth-century science.

Final arrangements were completed by M. Maurice Daumas (Conservatoire National des Arts et Métiers) and he proposed to some thirty participants, when they assembled at the Conservatoire, six sessions incorporating twenty-five speakers, who would each be allowed thirty minutes for reading and discussion of their announced memoir. This Procrustean principle, genially supervised by the sessional chairman, worked out surprisingly well. A provocative précis, giving time for a rapid-fire of terse, ready commentaries in French or English, often threw very helpful flash-lights on an unfamiliar subject.

The largest single groups attending were from France and the United States; but a useful British contingent accompanied the representatives of Eastern Germany, Eire, the Soviet Union, Sweden, Switzerland and Yugoslavia. Some others, including Douglas McKie, were unable to attend; but this was a notable, perhaps a unique concentration of interest and authority on the history of chemistry.

Reference to individual contributions must be invidious. It is in any event the intention of the sponsors to publish, under a separate cover, the proceedings of this colloquium. These again surveyed such pivotal pillars of progress as Boerhaave, Black, Priestley and Lavoisier. If perhaps too little was heard on Henry Cavendish and J. B. Richter, other figures such as Bergman, Crawford, Cullen, Geoffroi, Kirwan, de Morveau, Scheele, Tillet and Watt were exhibited in refreshing detail.

To the minority of professional chemists in attendance, there may have been something a whit rarefied in the attention given to Maupertuis and Gaspard Monge; but these were balanced by the stiffly practical consideration given by Academician Arthur Birembaut to early gas-manipulation or by F. Greenaway to contemporary analytical chemistry. None surely will forget the calm finality with which G. Kersaint buried the unsavoury legend of the treachery of Fourcroy to Lavoisier; Figourovskij straddling the difficulties of language and time in his projection of Lomonossov; Claude Secretan's cool, systematic appraisal of scientific personalities; the charming intervention of M. Braise, who now occupies the pharmacist's shop once served by Lavoisier's mentor, Guillaume-Francois Rouelle.

The formal sessions were extended to the lunch-table, where the exchangers of criticism, literature-reference and citation could communicate their ideas. There was also an exciting inspection of new acquisitions from Lavoisier's laboratory apparatus. (By an educative coincidence, Blériot's original biplane apparatus, in this jubilee year, was seen suspended from the ceiling of another room.) The foreign guests were permitted a privileged visit to the chemical archives of the Academy, an inspection of note-books by Lavoisier, and a glimpse of the meeting-rooms with their antique velvet, gilt frames, classic mouldings and individual microphones.

Personal and renewed impressions were of the countless historical trails that lead back to Newton and Boyle; the accepted importance of the chemistry of the Scottish universities in the eighteenth century; the long-delayed development of Stahlian doctrine in the hands of Macquer and Rouelle; the painstaking, sharp rescrutiny of the contributions by Lavoisier to chemical theory; the muddled yet stimulating notions of affinity; the factors and persons which foreran Dalton. An intimidating confusion of early schools and ideas, acquiring a laboured, significant sequence as the simple gases were isolated and understood; and there is still much historical material to arrange and digest before such studies bring full benefit.

In their development of chosen themes by address or in debate practically every speaker was mindful of the broader canvas against which the chemical feature must be examined. There was careful attention to contemporary physics, metallurgy and pharmacy; to economics and invention; even inevitably to politics, with some good-humoured interventions on delicate French and Irish situations. Here were specialists indeed, but specialists determined on an adequate setting of their studies even to "The Fifteen", the Mountain and Vinegar Hill. Humanism kept breaking in.

Much more than the usual formalities of praise must go to the organization of this conference. Informal, urbane, efficient, friendly, it produced the sadly impractical conviction that such meetings should be held more frequently, and always convened in Paris. Of still more lasting importance was the sense of community created. Historians of science and especially, one imagines, historians of chemistry must generally plough a somewhat lonely furrow, although there are seemingly a few sites of adequate appraisal and support. Here was the refreshment of friendly controversy, of actual and potential collaboration, and an atmosphere in which chemistry was seen as something even more than a magical technique, seen as a major factor in the climactic development of scientific method and modern science, as a bright particular strand in the culture pattern of Western mankind. On these last points the eighteenth century had already no doubts at all.

In some closing words of appreciation, Henry Guerlac used his graceful bilingualism to express our thankful sense of pleasure and profit from this occasion and hinted at a possible opportunity of meeting American colleagues in 1962. None commented on the very sparse German attendance or on the Italian absences which some later colloquium will surely find improved.

ANDREW KENT

FAST CHEMICAL REACTIONS IN SOLUTION

VERY many chemical reactions in solution proceed too rapidly to be studied by conventional techniques, which require times of at least several seconds for their manipulation. Slower reactions often involve several steps, all but one of which are fast. It is also generally true that the simplest and most fundamental reactions tend to be fast ones.

The development of electronic methods of detecting and recording has made it possible to record almost any physical parameter in a very short interval of time and therefore to carry out a chemical investigation. But, in order that such an observation shall be significant, it is necessary to initiate the chemical change in some way in a time which is short compared with the time of reaction. Methods of attaining this and some of the results obtained were the subject of a recent meeting organized by the Bunsengesellschaft für physikalische Chemie and the Max-Planck-Gesellschaft. The colloquium was held in Hahnenklee/Harz during September 14–17 and was attended by about one hundred and sixty scientists, of whom about ninety were from Germany, thirty from the United States, twelve from Britain and twenty-eight from other countries. Thirty papers were presented, covering practically every available method.

The first two days were devoted to a discussion of the fast-reaction methods and measuring techniques themselves. Historically the first method was the rapid-mixing method introduced by Hartridge and Roughton thirty-six years ago, and this was discussed by F. J. W. Roughton (University of Cambridge) with particular reference to the reactions of hæmoglobin, for which the method was first developed. It has been extended and modified in many ways, for example, by the introduction of stopped-flow techniques, and is useful for half-reaction times of about 1 millisecond or more. Flash photolysis, developed by Norrish and Porter, is a second generally useful method, and in describing this method G. Porter (University of Sheffield) pointed out that almost every type of reaction can be initiated photochemically, and that at present it is possible to measure bimolecular reaction-rates with velocity constants up to 10^{10} litres/mole/sec. and first-order constants up to 10^5 sec.$^{-1}$.

Q. H. Gibson (University of Sheffield) described how the stopped-flow method has been used to study the combination of hæmatin and carboxy-hæm with globin. Examples of the application of the flash photolysis method were given by H. Linschitz and J. Eloranta (Brandeis University), who used this method to study metal and hydrocarbon ion solutions, by H. T. Witt, who gave an extensive survey of the work which he and his co-workers have carried out on chlorophyll with particular reference to primary processes of photosynthesis, and also by E. W. Abrahamson (Syracuse University), who described work on the visual pigments.

Both mixing and flash-photolysis methods are applicable to systems which are far removed from equilibrium as well as to relaxation systems. Most of the other fast-reaction methods start with a system at equilibrium, displace the equilibrium slightly by changing some physical parameter and then observe the rate of relaxation to the new equilibrium position. Dr. M. Eigen and his colleagues at the Max Planck Institute for Physical Chemistry in Göttingen have been pioneers in this field, and a comprehensive review of relaxation methods and an account of the mathematical procedure and of the assumptions necessary to evaluate rate constants from the experimentally determined parameters was given by L. De Maeyer. Displacement can be either periodic or of the jump type, and temperature, pressure, field impulse and sound absorption are all used to produce the disturbance. K. Tamm (University of Göttingen) gave a survey of the use of the sonic relaxation method for rate measurements in water solutions. M. Eigen summarized many reactions which have been studied by relaxation methods/ The fastest reactions which are controlled by dif. fusion give rate-constants of about 10^{10} litres/mole-sec. for ionic reactions other than protonic where values of about 10^{11} litres/mole/sec. are obtained. The use of the ultrasonic technique to study association reactions and rotational isomerizations was outlined by Van W. Maier (University of Freiburg) and J. Lamb (University of London) respectively, and the application of the relaxation method to enzymatic reactions was discussed by R. A. Alberty and G. G. Hammes (University of Wisconsin).

A new and very powerful approach to fast reactions is provided by the methods of electron and nuclear magnetic resonance. A molecule, such as water or hydrogen peroxide with two equivalent protons, gives a single peak in its nuclear magnetic resonance spectrum. A mixture of water and hydrogen peroxide will give two separate peaks if there is no exchange between the protons of the two molecules. However, if there is a fast exchange of the protons, there will be only one peak as all the protons will then appear equivalent. For intermediate cases the exchange-rate constants may be calculated from the form of the spectra. The nuclear magnetic resonance method can be used for reaction times from 1 to 10^{-3} sec. Electron spin resonance spectra can be interpreted in an analogous way to determine reaction-rates with characteristic times in the range 10^{-4} to 10^{-9} sec.

The carbon dioxide – water equilibrium has been studied by A. Patterson and R. Ettinger (Yale University) using nuclear magnetic resonance methods. Patterson pointed out that two most interesting questions remain unanswered, namely, why is such a large proportion of carbon dioxide in solution present in dissolved but not hydrated form, and, secondly, why is the hydration reaction such a slow process. R. G. Pearson (Northwestern University) explained how paramagnetic ions in solution affect the line-shape and how line-width can be used to measure exchange rate constants for reactions of complex ions.

Methods which are not quite so general in their application include the fluorescence method—A. Weller (Technischer Hochschule, Stuttgart) described how it is used to measure the fast reactions of excited molecules—and electrochemical methods. R. Brdicka (University of Prague), Jiri Koryta (Academy of

Science, Prague) and others gave comprehensive reviews on the use of polarographic technique in the study of electrode reactions.

The transfer of electronic excitation energy between molecules is a special type of fast reaction which, in some cases, is so efficient that rate constants exceed those calculated for the maximum rate of a diffusion-controlled reaction using normal collision diameters. Prof. Th. Förster (Technischer Hochschule, Stuttgart) explained how the interaction in such cases occurs through dipole–dipole coupling so that energy transfer can take place over distances as great as 50 A. Two examples of the direct measurement of energy transfer processes were presented. S. S. Brody (University of Illinois) has used a small specially designed hydrogen lamp to produce a flash of a few millimicroseconds duration. He measured the delay between the flash and the fluorescence of chlorophyll in the chloroplast. By using suitable filters, other pigments apart from chlorophyll were made to absorb the light and the sensitized fluorescence of chlorophyll was studied. M. Burton and H. Dreeskamp (University of Notre Dame) were also able to resolve decay times of about 10^{-9} sec. A low-voltage X-ray tube was used to deliver an approximately square wave pulse of about 10^{-8} sec. duration with rise and fall times each about 10^{-9} sec. By this means the energy transfer from benzene to *p*-terphenyl was measured.

Papers by H. Eyring (University of Utah) and R. M. Noyes (University of Oregon) dealt with questions of solution structure and fast reactions. Noyes has developed a simple theory to predict the quantum yield for iodine dissociation from the values of the viscosity of the solvent, the sizes and masses of the fragments of dissociation, the energy of the bond that is broken and the energy of the absorbed quantum. The agreement between theory and experiment is remarkably good for such a simple model.

A very pleasant feature of the meeting was a visit to the Max Planck Institute in Göttingen, where a number of experiments on fast reactions in solution were demonstrated. This visit provided a unique opportunity to compare and contrast many of the methods discussed at the colloquium. The methods demonstrated included (1) flow methods, (2) relaxation methods—field impulse, temperature and pressure jump and sound absorption, (3) polarographic methods, and (4) the nuclear magnetic resonance method.

A lively discussion followed most of the sessions and was greatly facilitated by an excellent earphone translation system between the German and English languages. Most of us left the meeting feeling that, with such a wealth of excellent methods available, there is now practically no chemical reaction in which rate cannot be directly measured, and the 'instantaneous reaction' has gone for ever. It was also clear that reactions which proceed in times of less than one second are as numerous and at least as fundamentally important as those which allow a more leisurely approach to rate measurement.

G. Porter
F. Wilkinson

SWISS SOCIETY FOR NATURAL SCIENCES

THE one hundred and thirty-ninth annual meeting of the Swiss Society for Natural Sciences was held at Lausanne during September 11–13. The annual president, Prof. A. Girardet (Lausanne), opened it, with an address on "La science, le savant et le pharmacien", touching the history and psychology of pharmaceutical teaching, practice and research, which in Switzerland are the concern of the universities and medical authorities far more closely than in Britain in the general pattern of scientific and industrial development. He pointed out in conclusion the financial handicap implied by the present-day loss of monopoly for the sale of many types of drugs, which has forced the pharmacist to extend his business to non-pharmaceutical products to the detriment of his professional standing. Once the equals of the surgeon in the employ of the medical theorist, the pharmacist and pharmacologist have not fought their way to the same independence and supremacy as he has.

The first general meeting adjourned to St. Croix in the Jura Mountains, a geological and industrial district of considerable interest, in magnificent weather enjoyed with mixed feelings by the inhabitants, entirely dependent on wells for their water. Prof. A. de Muralt (Berne) reported on the first seven years of the Swiss National Fund for Scientific Research, State subsidized but remarkably free from State control, and available to Swiss organizations and individuals in the widest conceivable manner. Nuclear research has naturally come in for a large share, and its potentialities and limitations in Switzerland were discussed separately in more detail by Prof. P. Hüber (Basle), who has been most concerned with it.

The remainder of the general proceedings took place in the fine painted Aula of the University of Lausanne, adjoining the magnificently re-organized up-to-date library and the Museum of Fine Arts, where one of the hospitable receptions was held, all of them pleasantly devoid of any visible host or formality of any kind.

After an illuminating account by P. Muller, of the Paris Observatory, on "Le Pourquoi des Satellites Artificiels", and a masterly and moving survey, "La Science à la Recherche d'une Conscience", by the annual vice-president, Prof. D. Rivier (Lausanne), the day ended with a report by Dr. G. Chevallaz (syndic of Lausanne) on preparations in hand for the Swiss National Exhibition to be held in 1964 at Lausanne: in better circumstances, it is to be hoped, than its predecessors of 1914 in Berne and 1939 in Zurich.

Finally, at the third general meeting, Dr. F. Kaudewitz, of the Max Planck Institute of Tübingen, spoke on "Methoden, Denkweise und Ergebnisse der Erbforschung an Bakterien", with a sober simplicity, clarity and directness remarkable in so new and complex a domain; and the *sous-directeur* of the National Museum of Natural History at Paris, T. Dorst, under the title "La Signification des Galapagos pour l'Étude de l'Évolution", gave the latest findings and hypotheses on these unique islands.

The meeting was timed to coincide with the annual Comptoir Suisse at Lausanne, in order to give Swiss residents the advantage of return travel at single fare by means of an automatic ticket-stamping device inside the Comptoir, with a reduced entrance fee for members of the Society. The simultaneous disadvantage that hotels had to be booked long in advance kept away a certain number of members. Nevertheless, the sectional proceedings were on the whole as lively and well attended as ever, the Geophysical, Meteorological and Astronomical Section in particular being fortunate in having the director of the Swiss Meteorological Station, Prof. J. Lugeon, at hand in person after his extensive travels of recent years, partly in connexion with the International Geophysical Year and two of them to the North Pole, to report, with collaborators, on "Les Recoupements Radiogoniographiques des Grands Foyers Orageux de l'Hémisphere Nord depuis la Base Spitzberg-Suisse (4100 km.)" and "Comparaison des Régimes des Parasites Atmosphériques Polaires et Européens", and to give a preliminary report on measurements of atmospheric electricity and radioactivity of the air at Murchison Bay during the International Geophysical Year. The History Section was specially interesting with reports on the mathematical aspect of the Hippocratic Logos (Dr. Lichtentaeler, of Leysin), on Baldasar Heseler's eyewitness account of Vesalius's first public anatomy lecture at Bologna in 1540 (Dr. N. Mani, of Basle) and on the little-known mathematician and philosopher de Crousaz, of Lausanne, 1663–1750 (Dr. Ed. Fueter, of Wädenswil, ZH). Perhaps the Physics Section was the busiest, under the annual vice-president, Prof. D. Rivier, with twenty-eight papers mainly on metal, crystal and atomic physics.

Excursions after the meeting were organized for those interested in geology (3 days), mineralogy (2 days) and botany (2 days).

R. C. H. Tanner

SCIENCE AND THE LIBRARY SERVICES IN BRITAIN

THE programme of the annual conference of the Library Association usually includes two or three papers of some general scientific interest and, although that of the annual conference held at Torquay during September 22–25 was possibly of less scientific interest than usual, Prof. Lancelot Hogben's "Past, Present and Future of the Printed Page", Sir Sydney Roberts's "Reflections on the Roberts' Report", and Alderman F. J. Stott's "The Public Library as a Major Instrument of Education" are of wide interest. Besides this, Miss M. E. Wilden-Hart's "Observations on County and Municipal Practice" touched on various matters involved in the development of an adequate national library system whether for science and technology or in any other field. Earl Attlee's presidential address, succinct, wise and witty, illustrated the extent to which wide reading and the right handling of books are still positive constructive factors in modern life; and Lady Barlow discussed "Charles Darwin as Author".

Prof. Hogben addressed himself to three issues: Can other means of communication adequately replace the printed page? Will their rightful exploitation diminish the demand for the printed page relative to the size of the literate public? And how far does a plan of peaceful co-existence for the two call for a new programme of book design? On the first, Prof. Hogben remarked that 'adequately' was the operative word, and the freedom to turn the pages backwards or forwards to interpret the author's meaning in the light of previous statements or of what occurred later was an incentive to concentrated attention and acted as a brake on superficial assent. To regard reliance on mechanized instruction without reliance on the printed page as calamitous did not mean that the more extensive use of sound or television should be regarded with disfavour or that such extension would curtail the need for book learning. On the contrary, Prof. Hogben thought that we would have to exploit new means of instruction and that radio sound and television could solve the modern problem of shortage of talented teachers in a world of wider educational opportunities if—and only if—we could give it a curricula orientation entirely divorced from the prepossessions of the entertainment business. The exploitation of the newest visual aids did not mean that there would be need for fewer books in the near future. In some of the backward territories there would be vastly more readers if we could use the new means to speed up universal literacy, and where literacy was already well-nigh universal the new means could so expand the appetite for learning as to stimulate powerfully the output of new informative literature.

As regards the third question, while there was no reason to anticipate that the exploitation of the new means at our disposal would diminish the demand for the printed page, and ample reasons existed for believing that it would increase that demand, we could certainly forecast that it would increasingly change the content of what printed matter is in demand and set a new standard of design for books which satisfy an abiding demand. Here Prof. Hogben referred to the possibilities opened up by gearing issued and printed material into editions which could subserve two educational objectives, sentences of one line in length permitting repetition of the same message line by line in several languages. In the new era the first challenge to intellectual effort was how most expeditiously we could make available to all persons everywhere the printed page; the second was how to make everywhere accessible to all, both one and the same printed page and one and the same human voice. The third great intellectual challenge was how to disperse over an ever-widening and deepening understanding less of the mechanical techniques at our disposal than of the peculiarly human innovations.

Sir Sydney Roberts began by pointing out that the central question his Committee had been asked to consider was what changes, if any, should be made in the administrative arrangements of the public library service, and this included possible transferences of responsibility from one type of library authority to another. Although there were obviously two clashing views of the functions of a public library,

both were agreed that the public library service, by whatever type of authority it might be administered, was essentially a feature of local government. Nevertheless, the Committee was clear that provision of library facilities should cease to be a local option and should become a statutory duty with ultimate authority vested in the Minister of Education. Librarianship, however, was a highly specialized function, and in Sir Sydney's view a committee responsible for a public library should be an independent committee reporting direct to the library authority. Directing attention to the Committee's comment that size and efficiency did not necessarily march together, and its view that full opportunity—at least three years—should be given to any non-county borough or urban district to demonstrate its ability to fulfil the required conditions before any change was made in its library status, Sir Sydney said that the Committee had had before it the proposals for the new National Lending Library for Science and Technology, and its consideration of such matters had confirmed its misgivings about the imperfections of the reference sections in many libraries. Co-operation between libraries of an entirely different character and constitution, however, could best be achieved by individual negotiation, in the light of local conditions, between the libraries concerned. The main obstacle to higher efficiency in the working of the regional bureau, however, was finance, and the Committee was not prepared to advocate a standard pattern of administration over the whole country. Finally, after touching on the problems of book selection, financial and otherwise, Sir Sydney pointed out that the public libraries must take full account of the implications of the scientific revolution in which we live. They must stock both the technological text-books and reference books needed by their industrial and academic clients and they must make a judicious choice of those authors who attempt to explain the basic principles of scientific development to the non-scientific reader. They had also to recognize the sociological results of scientific discovery and development and meet adequately the recreational needs of leisure.

Alderman F. J. Stott, who began with a quotation from the report of President Eisenhower's Science Advisory Committee, "Education for the Age of Science", stressing that the aim of an educational system must always be the development of the individual so that he could live the fullest life with himself and the community, elaborated further Sir Sydney Roberts's closing remarks. If the scientific age challenged technologists to make a special effort to ensure that their education was not detrimental to their balanced development as individuals and challenged non-technical people to keep in touch with the new language and thoughts of science, it was essential for our national welfare that we should stay in communication with each other, and the channels of communication could only be secured by the universal enjoyment of liberal education. Education, however, was being required to devote more and more of its resources to vocational and technical training at the expense of wider values, and while at work more and more were called upon to do less and less thinking, in our ever-increasing leisure more and more were content to leave the planning of their leisure to the instruments of mass entertainment.

In this situation, in which the integrity and full flowering of the individual were threatened on all three counts, Alderman Stott thought that the public library had an important part to play. A survey of readers recently made in the Plymouth public libraries indicated that, whereas in Plymouth 33,000 scholars at primary, secondary and grammar schools spend 33·5 million hours in attendance, 66,000 readers of all ages devoted 36·5 million leisure hours to reading, of which 15 million were devoted to reading of a deliberately self-chosen, informative and educational character. On the provision of this service some £80,000 a year was spent, compared with about £2 million on formal education in primary and secondary schools, but Alderman Stott, of course, emphasized that on two-thirds to three-quarters of the population the public library services made no impact at all. He did not think it was simply a matter of increasing the provision for public library services but rather, as Lord Attlee had suggested, for encouraging children early in life to use and cherish books. There was also need for much more publicity and some attempt to meet the needs of individuals.

INDUSTRY AND HEALTH IN BRITAIN

THE annual report of the Chief Inspector of Factories for the year 1958 follows the new form adopted last year, which was very well received, and there is again a separate report on industrial health (Ministry of Labour and National Service. Pp. 95. (Cmnd. 810.) London: H.M. Stationery Office, 1959. 5*s*. 6*d*. net). While the number of reported accidents fell by 4 per cent, from 174,713 to 167,697, and was the lowest reported for any year since 1935, fatal accidents rose from 651 to 665. For factories alone the figure declined by 5 per cent to 143,099, of which 368 were fatal; but in building operations and works of engineering construction there were increases from 14,568 to 15,017 and from 1,924 to 2,329, respectively. The former included a considerable increase in fatalities due to falls, especially of materials, and this appears to be due to much demolition work being carried out by inexperienced personnel with little or no appreciation of the loading and stresses in various parts of the structure on which they are working. No figures are available for accident incidence rates of individual industries, although the report gives comparative frequency rates, based on voluntary returns from individual firms, and it is hoped to publish a table of incidence rates in next year's report. The distribution of accidents between the sexes and between young persons and adults was little changed, but the report points out that, in spite of the emphasis placed on training young persons entering industry, 9,838 young persons were involved in notifiable accidents and 8 of them were killed: in some industries it is still

true that the probability of an accident is greater for a young person than for an adult.

The Chief Inspector directs attention to the increase in accidents due to transport other than rail, where the 1958 figure was more than double that for 1948, and to the need for much more attention to problems of safety in the use of road transport within factories and other work-places: greater care, for example, might be exercised in selecting drivers and operators of such transport. Accidents associated with the use of power presses again increased in spite of great efforts to evolve better designs and methods of guarding, and greater attention to maintenance of these machines and their guards appears to be needed.

The new arrangement of the report gives prominence to the aspects of special interest to the scientist. There is a separate chapter on the problems encountered by the Inspectorate in relation to nuclear energy and ionizing radiations, which covers electrical instrumentation and the control of nuclear reactors, as well as the preparation and fabrication of special metals used in reactors, the preparation of enriched uranium, the handling and treatment of irradiated fuel rods and sealed and open sources of radiation. A chapter on fire prevention stresses the need to report dangerous occurrences which are not associated with personal injury, and attention is directed to the possibilities of eliminating sources of ignition by less hazardous arrangements as well as to the hazards associated with waste and with the handling of inflammable liquids even on a relatively small scale. The limitations of devices based on developments in modern telecommunications techniques are discussed and special attention is directed to the hazards associated with organic peroxides. Here and for the handling of mercury the report details a number of specific precautions, and the advantage of placing any potentially explosive plant, such as that for handling hydrogen, outside any occupied room and where explosion is unlikely to injure anyone if it occurs, is indicated. The scientific interest of the work of the Inspectorate is further illustrated by the list of inter-departmental committees on which inspectors have served, and the report records 407 inspectors of all grades in posts at the end of the year with vacancies for nine general, eleven chemical, eight engineering and two medical inspectors.

THE BRITISH FORESTRY COMMISSION

THIRTY-NINTH ANNUAL REPORT

As remarked in the opening sentence of this report*, by far the most important event of the year in British forestry was the statement on policy made in Parliament. In view of the developments and progress since the post-war policy was adopted in 1946, planting is to continue at approximately the current annual rate of 60,000 acres for another five years, after which a significant reduction, about a fifth, is envisaged, when the older plantations will be coming into full production at an age of around fifty years. There is no specific abandonment of the old national target of five million acres of productive woodland for Britain as a whole, though it is recognized that its strategic basis has altered considerably. No specific target is set for the planting of private woodlands, currently running at 32,000 acres annually, but the policy of encouraging planting and more intensive management is continued, the acreage grants being raised and somewhat modified. Action is also being taken to improve the marketing of produce from these private woodlands as it has long been recognized as unsatisfactory, with consequent discouragement to owners and potential investors. The increased grants were offered on condition that owners organized an effective association for marketing and connected purposes, and they have in fact now done this on a regional basis; at the same time, the warning is given that the grants are not to be viewed as a permanent subsidy, but only as an initial stimulus and help which should fairly soon become unnecessary. Felling licences will be continued, not to restrict felling to a predetermined quota, but to ensure acceptable felling practices and replanting where necessary.

* Forestry Commission. Thirty-ninth Annual Report of the Forestry Commissioners for the year ended 30th September 1958. Pp. 84+7 plates. (London: H.M. Stationery Office, 1959.) 5s. net.

Once again, difficulties in acquiring the necessary land for the annual planting programme constitute a major handicap, and the necessity of paying rather higher prices than in the past is accepted. It is hoped that more land will become available particularly in Scotland and Wales, where the expansion of forestry will provide diversification of employment and important social benefits.

Although one of the most direct benefits of increased timber production in Great Britain should obviously be a gradual reduction of imports which run at present at around £170 million per annum, we should now be entering a phase of quite considerable development of wood-based industries; this should more than compensate for some drop in consumption of unprocessed wood, such as has occurred with pit-props and railway timbers. The opening of a large hardwood pulp mill on the Severn estuary is an important event and is being followed by similar developments in various parts of Great Britain. The possibilities of small-scale pulping are under special consideration and seem quite promising.

Expenditure on research now stands at more than £300,000 annually. A short summary of progress in the several branches of the work is included in this report though a full account is published separately. Research facilities have been greatly improved during the year by the provision of a large new building which was formally opened in July.

Reviewing the situation as a whole, it is clear that the decision taken forty years ago to remedy the extremely unsatisfactory forestry position in Britain has been acted on with a measure of success that rarely attends such long-term ventures, and we are now rapidly approaching the time when the returns, both direct and indirect, will be coming in at a rapidly increasing rate: already sales of produce are bringing in more than £3,000,000 in the year.

ULTRASONIC BARIUM TITANATE ADHESION AND PASTE TRANSDUCERS

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PRELIMINARY observations on the behaviour of two new types of transducer will be described to emphasize the possible importance of these generators. They offer possibilities particularly in overcoming the following problems which arise in applying transducers available at present.

(1) For the generation of elastic waves in solids at very high frequencies, a quartz crystal of a thickness of 0·1 mm.—having a fundamental resonance frequency of approximately 30 Mc./s.—is, due to fragility, the smallest thickness that can be used in practice.

(2) The coupling layer between the transducer crystal and the test object should be as thin as possible, preferably less than $\lambda/20$. This again sets a limit for high-frequency applications.

Exciting a crystal in its harmonic modes is not always a reliable method and does not solve the coupling problem. The importance of extending the frequency-range is summarized in ref. 1.

(3) In addition, in non-destructive testing, the degree of coupling between an ultrasonic transducer and object is unknown[2]. A firm and stable contact is desirable, but is especially difficult to obtain at rough and curved surfaces.

(4) The resonance frequency of a crystal transducer is not variable—a disadvantage in some applications.

New desirable transducer types and manufacturing methods are mentioned in ref. 3.

A transducer produced on the surface, forming an integral part with the test block, would avoid the handling of extremely thin crystals and the necessity of providing a coupling layer. This is obviously not possible with conventional barium titanate transducers[4], because during the production a temperature of 1,350° C. is required which cannot be tolerated for most test objects.

The Barium Titanate Adhesion Transducer

Barium titanate powder was mixed with a liquid ethoxyline and heated to remove moisture. After allowing this mixture to cool to a few degrees above room temperature, a hardener was added and the mixture was cast on the surface of a metal block (Fig. 1). A ring made from an electrical insulating material of the same thickness as the desired ultrasonic transducer was placed on the surface of the metal block forming a casting mould for the mixture. Immediately after casting, while the mixture was still in a 'plastic' stage, a flat metal disk was placed on top of the ring. The disk and the metal block form the electrodes and two defining surfaces of the mould. A high-voltage direct current source was connected through a resistor to polarize the transducer, while simultaneously the electrical output of a conventional ultrasonic reflectoscope[5] was applied via a condenser. The reflectoscope transmitted short electrical pulses to the transducer cast. When the cast became operative as a transducer its performance could be deduced by observing the pulses reflected from the far end of the test block. The metal disk and the casting mould ring were coated with a thin layer of a silicone paste before assembly. It was found that this was sufficient to isolate the elastic vibrations of the transducer from the top electrode. By thorough cleaning of the metal surface of the test block an intimate elastic coupling to the case was obtained. The unit was heated to approximately 135° C. and allowed to cool to room temperature, while the high-voltage source and the reflectoscope were still connected. During this operation the mixture became a solid layer in firm contact with the test block, hence the name adhesion transducer.

The reflexion from the far end of the test object was observed and showed an increase in amplitude with decrease in temperature. The behaviour was tested by applying the pulses at carrier frequencies 0·5, 1, 2·5 and 5 Mc./s., which were available from the reflectoscope, to the same adhesion transducer.

The majority of the subsequent tests were carried out at 2·5 Mc./s. with a pulse-length of 1 μsec. The temperature cycle was repeated several times and the same result as described above was obtained. On heating a polarized adhesion transducer while the direct voltage source was disconnected, a decrease

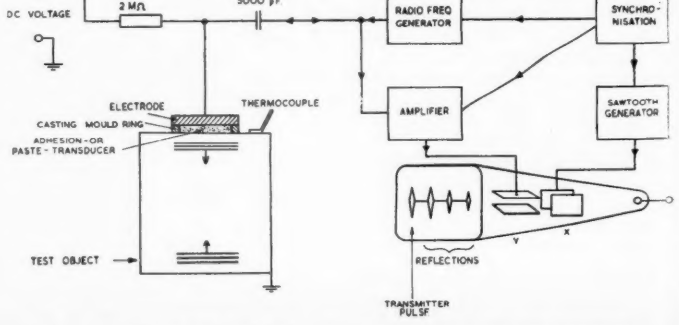

Fig. 1. Production and polarization of the adhesion and the paste transducer

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of the amplitude of the reflexion was observed. If the temperature exceeded 135° C. the amplitude dropped to zero and the transducer was depolarized. On heating a polarized transducer without the d.c. field to a maximum temperature of, say, 120° C., which is below the Curie temperature of conventional barium titanate transducers, its piezo-electric properties recover after cooling down but the amplitude of the reflexion does not increase to the original value.

While the optimum composition of the mixture from which the adhesion transducer was made is still being investigated, an attempt has been made to determine the effect of different barium titanate powder sizes. One problem for further investigation would be to test the expected usefulness of the adhesion transducer as a generator for high-frequency elastic waves. Since it can be expected that the relation of wave-length to grain size will be important in determining high-frequency limitations, the following tests were carried out. Because no electronic apparatus with higher carrier frequencies than those already mentioned was available, coarser powder was used while the frequency was held constant in an effort to obtain an estimate of the effectiveness of fine powder at correspondingly higher frequencies. Powder diameters between $d = 50$ μm. and 500 μm. in five grades were available. The velocity of sound of the transducer for compressional waves was found to be $2{\cdot}7 \times 10^5$ cm./sec. The following measurements were also made at a carrier frequency of 2·5 Mc./s. The corresponding ratios of the wave-length in the transducer to the powder diameter d were $21 > \lambda/d > 2{\cdot}1$. No significant differences in the observed reflexion amplitudes were observed, the coarse grain transducer ($\lambda/d = 2{\cdot}1$) seeming if anything to be slightly more effective.

The electrical impedance of the adhesion transducer was found to be considerably higher and the dielectric constant considerably smaller (ε_r approximately 7) than the corresponding values for conventionally made barium titanate transducers.

The adhesion transducer as described was hardened for a few minutes at a temperature above 100° C. The polarization of the transducer made from fine powder was effective only if the temperature exceeded 135° C. On the other hand, the use of coarse powder in making adhesion transducers allows an important modification of the manufacturing procedure. The remarkable effect was observed that they could be polarized by application of a d.c. field at room temperature, while the transducers were still in the 'plastic' stage forming a thick paste. The hardening can take place at room temperature, but hours or days are required, the actual time depending on the mixing ratios, the physical dimensions, and ambient temperature. This modification is important where test objects cannot tolerate a temperature of 135° C. After the transducer has hardened to a solid layer, it can be de- or re-polarized only above 135° C. in the manner already described.

The Barium Titanate Paste Transducer

The procedure for manufacturing a paste transducer is identical with that described at the end of the last paragraph, except that no hardener is added to the mixture and only grain sizes above a certain value are useful. The mixture remains a paste independent of temperature and time. It is polarized at room temperature. The first polarization curve and the hysteresis loop of the de- and re-polarized curve are shown in Fig. 2.

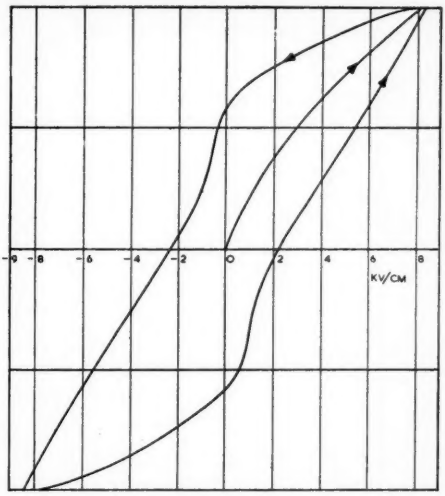

Fig. 2. Ferro-electric hysteresis of a paste transducer. Amplitude in percentage of the reflexion from the far end of the attached test block as a function of the polarization field in kV./cm. Measured at 2·5 Mc./s., and 25° C.

The paste transducer can be de-polarized (i) by means of stirring the paste ; (ii) by means of a d.c. field of an appropriate negative value (see Fig. 2). The behaviour of these paste transducers was totally unexpected in that unlike conventionally made barium titanate transducers and the adhesion transducer it was found still to operate above 135° C. Even at 200° C. a reflexion amplitude of 66 per cent and at 300° C. of 40 per cent of the value measured at room temperature was observed. The piezo-electric performance of the paste transducer is also less dependent on temperature than that of the adhesion transducer.

A paste transducer made from finer powder was found to be less effective, and for the finest available size no piezo-electric effect was measured. An obvious advantage of the transducer in paste form would be that by altering the distance between the upper disk electrode and the test block, using a casting mould ring of an elastic material, the transducer could be tuned to $\lambda/2$ for a given frequency of the electrical oscillator.

Stability of Performance of the Transducers immediately after Polarization

After removing the polarization d.c. voltage on a paste transducer it was found that the amplitude of the reflected pulse decreased from 100 to 63 per cent in approximately 10 sec., and to 60 per cent (as shown in Fig. 2) during the subsequent minute, after which it remained practically constant. No further ageing could be observed over a period of two weeks.

By adding a hardener, as described for the production of the solid adhesion transducer, a hardening time of several days can be arranged using a suitable low temperature. The following remarks concern a transducer with a final hardening time of two days. Immediately after the hardener was added the same behaviour as mentioned above for the paste transducer was observed. Removal of the polarization

field 2 hr. after the addition of hardener increased the 100 to 63 per cent reflexion decay-time mentioned previously, from 10 sec. to several minutes. In later stages between the 'plastic' and solid state this time may further increase to several hours. No decay was observed after the transducer layer had become a solid.

Some Aspects of the Application of the New Transducers for the Non-destructive Testing Technique

A useful application of the transducers is in the non-destructive testing of objects having a rough or curved surface. Unlike conventional transducer probes they are in direct contact with the object. Furthermore, no damping block on the back of the crystal is required for increasing the frequency bandwidth, as is necessary in conventional transducers, because the mechanical damping of most test objects is sufficient even at a rough surface. Detuning of the transducer, having a band-width, caused by a rough or irregular surface, is not of importance in practice; this can be corrected by re-tuning in the case of the paste transducer, as already mentioned above. At rough surfaces a reflectoscope, operating with the new transducers, should be capable of detecting cracks with a smaller area or at a greater distance from the transducer than is possible with a conventional apparatus. The so-called 'dead zone' would also be smaller because the transducer bandwidth is wider and therefore shorter pulses can be applied.

Theoretical investigation and further experimental work are continuing to (*a*) explain the observed ferro-electric and dielectric behaviour; (*b*) extend the frequency-range of the transducers by considering, for example, possible spray and evaporation techniques for obtaining physically thin layers.

I thank Mr. J. D. N. van Wyk of the National Physical Research Laboratory for valuable discussions, and Mr. E. R. Schmidt for preparing the barium titanate powder.

[1] Morse, R. W., *J. Acoust. Soc. Amer.*, **30**, 380 (1958).
[2] Kelman, D. M., Amer. Soc. for Testing Materials, Tech. Pub. No. 101, pp. 90–101 (1949).
[3] Hunt, F. V., *J. Acoust. Soc. Amer.*, **30**, 375 (1953).
[4] Mason, W. P., *Bell Lab. Record*, **27**, 235 (1949).
[5] Lutsch, A., *Arch. tech. Messen*, **91**, 193 (1954).

INCORPORATION OF N-METHYL CARBON OF MONOMETHYL-AMINOAZOBENZENE INTO POLAR DYES

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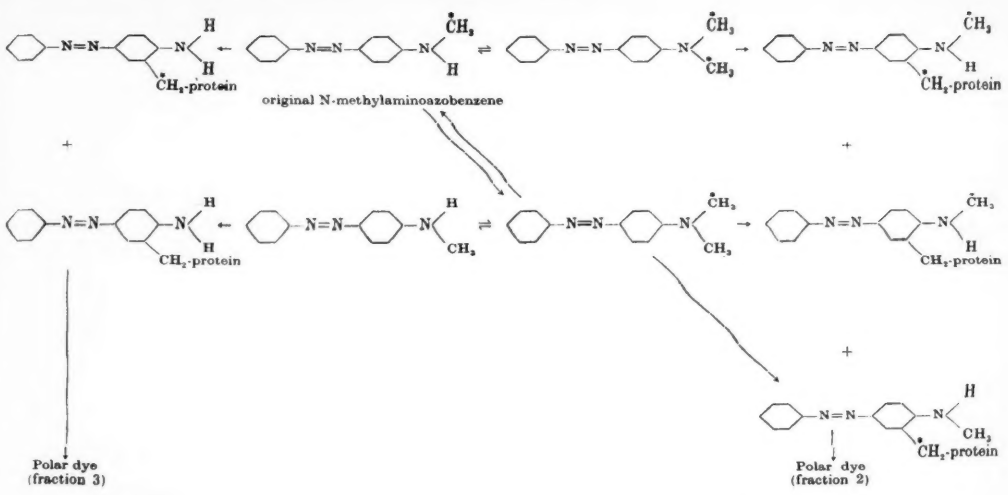
THE chemical binding between carcinogenic aminoazo dyes such as dimethylaminoazobenzene and cellular protein of rat's liver was first discovered by the Millers[1], who then proposed the protein deletion hypothesis as the basis of chemical carcinogenesis.

The chemical nature of the binding, however, has not been sufficiently investigated, due to the difficulty of purifying the polar dyes from the hydrolysate of the dye-bound liver proteins as well as the small amounts available. Terayama *et al.* recently studied the purification of the natural polar dyes from the hydrolysates of the dye-bound rat liver proteins[2,3] and discovered the presence of at least two types of polar dyes, depending on the nature of the amino group of the dye moiety, one having the primary (fraction 3) and the other having the secondary amino group (fraction 2)[3,4]. They also showed that the binding is at position 3, ortho to the amino group, in the dye moiety of polar dyes prepared from rat liver after the administration of dimethylaminoazobenzene or N-methylaminoazobenzene[3]. Evidence for position 3 binding is the remarkable spectral change (disappearance of the 500–520 mμ absorption in acid media) shown by the polar dyes after the 4 position amino group of the polar dyes is methylated up to the dimethylamino group with a mixture of formaldehyde and formic acid. The binding at position 4-amino group[5] was excluded so far as the acid-stable polar dyes (fractions 3 and 2) derived from dimethylaminoazobenzene or N-methylaminoazobenzene are concerned. This was confirmed by the result of the comparison with the synthetic acid-labile polar dyes which have the amino-acid substitution at the amino group (Mannich base)[3,6]. The amino-acid which is responsible to the binding in the protein moiety was assumed to be tyrosine mainly from the strong Folin–Ciocalteau's reaction, ultra-violet absorption and stability against acid treatment of the polar dyes[3]. The bridge to bind the dye to the tyrosine residue was thought tentatively to be a methylene[3] or oxymethylene, $-OCH_2-$ (unpublished work), group mainly on account of the necessity for at least one N-methyl group in the potent carcinogenic aminoazobenzene dyes as indicated by the Millers[5,7]. Direct evidence in support of the presence of a methylene (or oxymethylene) bridge has, however, been lacking. The present communication gives experimental support for the incorporation of the N-methyl group of N-methylaminoazobenzene into the polar dyes at a position other than the 4-amino group of the dye moiety.

N-^{14}C-methylaminoazobenzene was administered to rats and the polar dyes were prepared, fractionated and measured by the radioactivity as well as the optical absorption. 25 mgm. of the labelled compound in 2 ml. of olive oil was administered intragastrically under ether anæsthesia. The rats thus treated were killed 3 days later, livers removed and the polar dyes prepared as described in earlier papers. The partially purified mixture of polar dyes in 2 *N* hydrochloric acid was submitted to '*IRC*-50' (*H* type) column chromatography. The dyes were adsorbed at the top of the column, washed with



Scheme for the formation of protein-dye *in vivo* upon the administration of labelled N-methylaminoazobenzene

2 *N* hydrochloric acid, water and 30 per cent alcohol successively and then eluted with 60 per cent alcohol. Three fractions were separated. The portion not removed with 60 per cent alcohol was then eluted with 2 *N* ammonia (fraction 4). Fraction 1 seems to be contaminated with a small amount of non-polar dye. Fraction 2, a major component, having absorption maximum at 522 mμ in 2 *N* hydrochloric acid in 50 per cent alcohol, is the polar dye with the secondary amino group, and fraction 3, a minor component, having absorption maximum at 514 mμ in the same solution, is the polar dye with the primary amino group. Fraction 4 seems to be a mixture of the polar dyes with the longer polypeptide chains and can be further fractionated after being hydrolysed again. The acid-stable fractions 2 and 3 were re-chromatographed. The radioactivity and the optical density measurements for the portions of the eluates are closely paralleled.

The specific radioactivities of the purified polar dyes (fractions 2 and 3) are listed in Table 1. Since we do not know the exact molar extinction coefficients of the polar dyes, the amount of the fraction 2 polar dye is expressed in terms of N-methylaminoazobenzene or 3-methyl-N-methylaminoazobenzene, and for the fraction 3 polar dye in terms of aminoazobenzene or 3-methylaminoazobenzene tentatively. It is very interesting to see that the polar dye having the primary amino group, fraction 3, still holds an appreciable amount of the radioactivity derived from the N-methyl group of the original N-methylaminoazobenzene. This demonstrates clearly that the N-methyl group of the latter is certainly incorporated into the polar dye at a position different from the position 4-amino group. The specific activity of fraction 2 polar dye seems to be greater than that of fraction 3, the original radioactive N-methylaminoazobenzene being intermediate. Although the comparison of radioactivity in a strictly quantitative sense should be suspended until the exact molar extinctions of the polar dyes are elucidated, the result obtained here might be explained by assuming that the N-methylaminoazobenzene administered is further methylated *in vivo*, as indicated by the Millers[8,9], via the methyl pool, which is partly endogenous and partly derived from N-methyl metabolism of the radioactive N-methylaminoazobenzene, thus forming doubly labelled dimethylaminoazobenzene, which is then metabolized in the liver. It seems reasonable to assume that fraction 2 polar dye is derived from the latter thus formed *in vivo* and the fraction 3 polar dye from N-methylaminoazobenzene, the radioactivity of which might be somewhat diminished *in vivo* as the result of the intracellular demethylation and methylation reactions, as shown in the accompanying scheme.

Table 1 (*a*). Yield of Polar Dyes

From five rats, 25 mgm. N-methylaminoazobenzene in 2 ml. olive oil per rat

Polar dyes	Yield (γ) (in terms of N-methylaminoazobenzene)
Total polar dye, unfractionated	72·5
Fraction 1*	15·3
Fraction 2	30·7
Fraction 3	16·9
Fraction 4	5·4

* A part of fraction 2 is mixed in this fraction

Table 1 (*b*). Specific Radioactivity of the Purified Polar Dyes

Polar dyes	Specific radioactivity (e.p.m./γ)	
Fraction 2	580 ± 27	(in terms of N-methylaminoazobenzene)
	c. 580	(in terms of 3-methyl-N-methylaminoazobenzene)
Fraction 3	224 ± 18	(in terms of aminoazobenzene)
	c. 270	(in terms of 3-methyl-N-methylaminoazobenzene)
N-^{14}C-methylaminoazobenzene (administered)	430 ± 22	

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[1] Miller, E. C., and Miller, J. A., *Cancer Res.*, **7**, 468 (1947).
[2] Kusama, K., and Terayama, H., *Gann*, **48**, 181 (1957).
[3] Terayama, H., Kusama, K., Teruya, K., Kuroda, S., and Nakayama, T., *Gann*, **49**, 85 (1958).
[4] Terayama, H., Kusama, K., and Aoki, T., *Gann*, **49**, 97 (1958).
[5] Miller, E. C., and Miller, J. A., "Adv. in Cancer Res.", **1**, 339 (1953).
[6] Kusama, K., Terayama, H., and Teruya, K., *Gann*, **49**, 79 (1958).
[7] Miller, J. A., and Miller, E. C., *J. Exp. Med.*, **87**, 139 (1948).
[8] Miller, J. A., Miller, E. C., and Baumann, C. A., *Cancer Res.*, **5**, 162 (1945).
[9] Miller, E. C., and Baumann, C. A., *Cancer Res.*, **6**, 289 (1946).

OXYGEN-CONTAINING FUNCTIONAL GROUPS IN THE ORGANIC MATTER OF A PODZOL SOIL

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IT has long been recognized that there is a close connexion between the mobilization and immobilization of metals and that of organic matter in the development of podzol soils. The mechanism involved remains obscure, largely because little is known about the chemical constitution of the organic material(s) moving from the *Ao* through the *Ae* into the *B* horizon of the profile. It seems reasonable to assume that the organo-metallic reactions involved depend on the nature and numbers of functional groups. For this reason, a systematic study was undertaken of such groups in the organic matter of the *Ao* and *Bh* horizons of the Armadale soil, a poorly drained podzol from Prince Edward Island[1]. This report deals only with oxygen-containing functional groups.

The organic matter from both horizons was extracted with 0·5 *N* sodium hydroxide solution. Parallel extractions were made under air and under nitrogen in order to evaluate possible effects of air-oxidation[2]. Following extraction, the organic matter of the *Ao* horizon was dialysed exhaustively against distilled water, while that of the *Bh* horizon was passed over 'Amberlite *IR*-120' exchange resin in order to remove most of the inorganic cations. Both extracts were then concentrated to near dryness on the steam-bath and finally dried at 100° C. under nitrogen. Where extractions were made under nitrogen, the most stringent precautions were taken to exclude air during extraction and purification. Wherever possible, the analytical data were checked by independent methods. Total acidity was measured by baryta absorption under nitrogen[3] and carboxyl groups by reaction with calcium acetate and with iodate–iodide[4]. Total hydroxyls were determined by acetylation[4,5] and phenolic hydroxyls by reaction with barium hydroxide[6] and by a modified Ubaldini procedure[7]. All hydroxyl determinations were made under nitrogen. Carbonyl groups were measured by the method of Brooks *et al.*[8] and methoxyls by Zeisel's method[9]. Ultimate analyses were done by dry-combustion. Infra-red spectra were recorded on a Perkin–Elmer Model 21 spectrophotometer, using potassium bromide pellets[10].

Irrespective of whether extractions were carried out under nitrogen or air, the material from the *Ao* was higher in carbon, hydrogen and nitrogen but lower in sulphur and oxygen than that from the *Bh* horizon (Table 1). A comparison of the results for functional groups in the *Ao* and *Bh* materials extracted under nitrogen showed that: (*a*) the *Bh* material was considerably more acidic, due entirely to the presence of more carboxyl groups; (*b*) the *Bh* organic matter was higher in total and consequently alcoholic hydroxyls since the phenolic hydroxyl content of both materials was similar; (*c*) there was little difference in the carbonyl content, and (*d*) the *Ao* organic matter contained more methoxyl groups (possibly because of the presence of undecomposed lignin).

Table 1. Analytical Characteristics

Elementary composition (per cent) (dry ash-free basis)	*Ao* material extracted		*Bh* material extracted	
	Under N	Under air	Under N	Under air
Carbon	56·92	57·16	50·22	49·50
Hydrogen	5·20	5·30	3·74	3·62
Nitrogen	2·34	1·87	0·72	0·67
Sulphur	0·70	0·68	1·30	1·33
Oxygen (by difference)	34·84	34·99	44·02	44·88
Ash (dry basis)	8·87	8·25	3·02	3·85
Functional groups (m.-equiv. per gm. dry ash-free organic matter)				
Total acidity	4·7	4·7	11·4	11·5
Carboxyl	1·5	1·9	8·2	8·6
Total hydroxyl	3·2	6·0	5·0	5·1
Phenolic hydroxyl	3·2	2·8	3·2	2·9
Alcoholic hydroxyl (total minus phenolic)	0	3·2	1·8	2·2
Carbonyl	6·0	3·6	6·2	5·5
Methoxyl	0·4	0·4	0·2	0·2

The results for total and alcoholic hydroxyls (difference between total and phenolic) may be high, since total hydroxyls were determined by acetylation with acetic anhydride. This reagent[11], besides reacting with hydroxyls of primary and secondary alcohols and of phenols, also reacts with sulphydryl groups and primary and secondary amines. What proportion of the total nitrogen and sulphur is present as such groups is not known.

The most important changes in the distribution of functional groups when the organic matter was extracted under air (in contrast with nitrogen) were the following: (*a*) a decrease in carbonyl and an increase in alcoholic hydroxyl groups suggesting, in part, some form of keto-enol transformation; (*b*) a small increase in carboxyls and a concomitant equivalent decrease in phenolic hydroxyls. Durie and Sternhell[12] found a similar 1 : 1 correspondence between the decrease in phenolic hydroxyls and the increase in carboxyls on oxidizing brown coal in alkaline solution in the presence of oxygen. They concluded that the increase in carboxyl groups arose from phenolic groups.

There were no changes in the total acidity and methoxyl content of the organic matter whether extracted under air or under nitrogen.

Table 2 shows that whereas all the oxygen of the *Bh* organic matter was found in the functional groups, only 60 per cent of the oxygen of the *Ao* material

Table 2. Distribution of Oxygen in Functional Groups

	O as COOH / Total O	O as OH / Total O	O as CO / Total O	O as CH_3O / Total O	O accounted for / Total O
Ao material extracted under nitrogen	0·14	0·15	0·28	0·02	0·59
Ao ,, ,, ,, air	0·15	0·27	0·16	0·02	0·60
Bh ,, ,, ,, nitrogen	0·60	0·18	0·23	0·01	1·02
Bh ,, ,, ,, air	0·61	0·18	0·20	0·01	1·00

Table 3. Major Bands and Relative Absorbances in the Infra-red of Extracted Organic Matter

Frequency (cm.$^{-1}$)	Relative absorbance *Ao* organic matter	*Bh* organic matter	Interpretation*
3,380	strong	strong	hydrogen bonded —OH and bonded —NH groups
2,910	medium	shoulder	aliphatic C—H stretching vibrations
2,840	medium	0	" " "
2,600	shoulder	shoulder	carboxyl " "
1,720	shoulder	strong	carboxylic carbonyl
1,620	strong	strong	joint interaction of hydroxyl and carboxyl with carbonyl (ref. 13), also possibly carboxylic groups associated with metals so as to give the carboxylate structure (ref. 11)
1,450	strong	0	CH_3 and/or CH_2 in plane deformation vibrations
1,400	0	medium	carboxylate
1,250	weak	0	phenoxy C—O
1,200	0	medium	carboxyl
1,030	medium	0	Si—O of silica due to the presence of clay

* After Bellamy (ref. 14) unless stated otherwise

was similarly distributed. The remaining 40 per cent was probably present as ring and/or ether oxygen. Unfortunately, all systematic errors which occurred in the determination of the other oxygen groups are accumulated in this figure (O accounted for).

The information from infra-red absorption spectra (Table 3) confirmed the chemical evidence with regard to the presence of the major oxygen-containing functional groups. In addition, the infra-red spectra suggested that the *Ao* material was more aliphatic in nature than the *Bh* material. There were no detectable differences in the infra-red spectra whether the organic matter was extracted under nitrogen or air.

The major functional groups (carboxyls, hydroxyls and carbonyls) found in the organic matter of both horizons are known to react with metals such as iron and aluminium. Therefore, the presence of large numbers of such groups, especially in the organic matter of the *Bh* horizon, offers a partial explanation for the observed reactivity to metals of this type of organic matter during the formation of podzol soils.

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[1] Schnitzer, M., and Wright, J. R., *Canad. J. Soil. Sci.*, **37**, 89 (1957).
[2] Bremner, J. M., *J. Soil. Sci.*, **1**, 198 (1950).
[3] Brooks, J. D., and Sternhell, S., *Austral. J. App. Sci.*, **8**, 206 (1957).
[4] Blom, L., Edelhausen, L., and Van Krevelen, D. W., *Fuel*, **36**, 135 (1957).
[5] Ogg, C. L., Porter, W. L., and Willits, C. O., *Indust. Eng. Chem., Anal. Ed.*, **17**, 394 (1945).
[6] Ihnatowicz, A., Prace Glownego Inst. Gorn. Kommun., No. 125, (1952).
[7] Mukherjee, P. N., Bhownik, J. N., and Lahiri, A., *Chem. and Indust.*, 633 (1957).
[8] Brooks, J. D., Durie, R. A., and Sternhell, S., *Austral. J. App. Sci.*, **9**, 303 (1958).
[9] Brauns, F. E., "The Chemistry of Lignin" (Intersci. Pub., New York, 1952).
[10] Schnitzer, M., Shearer, D. A., and Wright, J. R., *Soil Sci.*, **87**, 252 (1959).
[11] De Walt, C. W., and Glenn, R. A., *Anal. Chem.*, **24**, 1789 (1952).
[12] Durie, R. A., and Sternhell, S., *Austral. J. App. Sci.*, **9**, 360 (1958).
[13] Brooks, J. D., Durie, R. A., and Sternhell, S., *Austral. J. App. Sci.*, **9**, 63 (1958).
[14] Bellamy, L. J., "The Infra-red Spectra of Complex Molecules" (John Wiley and Sons, New York, 1956).

ACTIVE PRINCIPLES OF THE NEUROHYPOPHYSIS OF COLD-BLOODED VERTEBRATES

Chromatographic and Biological Characteristics of Fish and Frog Neurohypophysial Extracts

THE neurohypophyses of all mammals so far investigated contain oxytocin and arginine vasopressin, with the exception of that of the pig, which contains oxytocin and lysine vasopressin. There are some indications, however, that the active principles in the glands of other vertebrate classes may differ from the mammalian peptides. For example, it has been shown[1] that, in frogs kept in water, the increase in weight produced by the injection of the extract of a single frog pituitary is matched by about 1,000 mU. mammalian oxytocin but that, assayed on a mammalian uterus, a frog gland contains only about 40 mU. oxytocic activity. A similar difference in the ratio of activities has been shown by Sawyer[2] in the bullfrog.

In view of these indications, it was decided to investigate further the active principles in non-mammalian neurohypophysial extracts. The pituitary glands of three species of fish (trout, cod and pollock) and of frogs (*R. temporaria*) were used. Extracts of these glands produced oxytocic, vasopressor, antidiuretic, milk ejection and frog water-balance effects. The oxytocic and vasopressor activities were completely abolished by treatment with sodium thioglycollate. The resistance of the other activities to such treatment has, as yet, not been investigated.

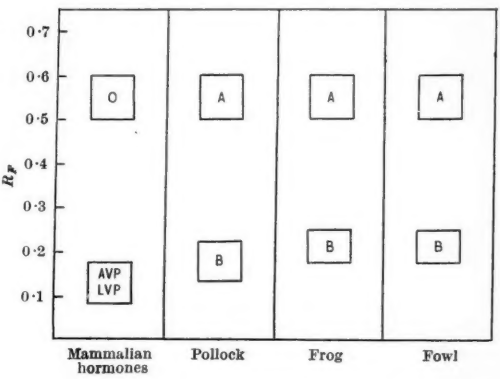

Fig. 1. Descending paper chromatograms of acetic acid extracts of mammalian and non-mammalian pituitary glands. The squares represent areas from which active substances could be eluted. Oxytocin (*O*), arginine vasopressin (*AVP*), lysine vasopressin (*LVP*), fast-moving oxytocic activity (*A*) and slow-moving oxytocic activity (*B*)

When subjected to descending paper chromatography in butanol/acetic acid/water (4 : 1 : 5) and stained by the method of Heller and Lederis[2], eluates containing oxytocic activity were obtained from two regions of the paper (Fig. 1). Eluate *A*, which was obtained from the same region as mammalian oxytocin, contained only a trace of antidiuretic activity and no pressor activity could be detected. Thus, almost all the pressor and antidiuretic activity of the original extract was found in eluate *B*. Both eluates caused milk ejection in the lactating rabbit and an increased uptake of water in frogs. As Fig. 2 shows, however, the frog water-balance effect of eluate *B* was much greater than that of eluate *A*.

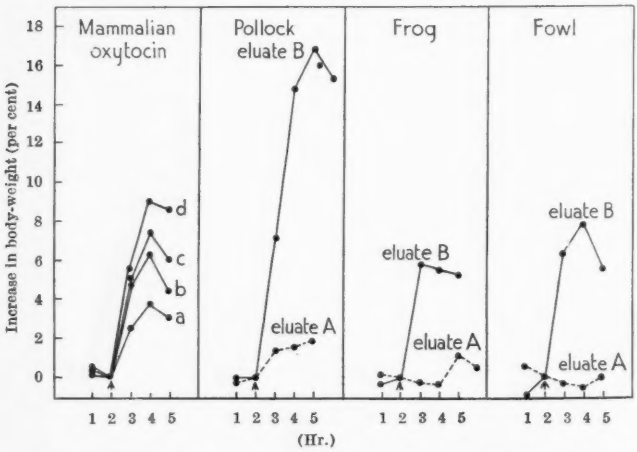

Fig. 2. Effects of eluates *A* and *B* (see Fig. 1) on frog water-balance. The volumes of eluates injected per frog had the following activity as assayed against synthetic oxytocin on the isolated rat uterus: Pollock, eluate *A*, 76 mU.; eluate *B*, 101 mU. Frog, both eluates, 7·5 mU. Fowl, both eluates, 12·3 mU. (*a*) 100; (*b*) 300; (*c*) 600; (*d*) 900 mU. oxytocin. Injections at arrow. The results shown are the mean responses of five or six frogs

It would seem, therefore, that the pituitaries of the three teleosts (two sea-water and one fresh-water species) and the amphibian investigated contain at least two oxytocic hormones, one of which behaves chromatographically and pharmacologically much like mammalian oxytocin. The other, which differs from the vasopressins in its marked oxytocic and frog water-balance activity, also seems to occur in fowl posterior pituitaries (Fig. 1). These results raise the interesting possibility that the hypothalamus of the vertebrate classes mentioned elaborates oxytocin and another active peptide not present in mammalian glands.

We should like to thank Dr. Grace Pickford for kindly supplying the pollock pituitary powder used in these experiments.

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[1] Heller, H., *J. Physiol.*, **100**, 125 (1941).
[2] Sawyer, W. H., in "The Neurohypophysis", edit. Heller, H., 171 (Butterworths, London, 1957).
[3] Heller, H., and Lederis, K., *Nature*, **182**, 1231 (1958).

Pharmacological Evidence for the Presence of Arginine Vasotocin and Oxytocin in Neurohypophysial Extracts from Cold-blooded Vertebrates

Munsick, Sawyer and van Dyke[1] have reported that chicken neurohypophysial extracts contain two active polypeptides. One appears to be oxytocin. A second peptide, present in greater quantity, has pharmacological and chromatographic characteristics indistinguishable from those of arginine vasotocin.

Arginine vasotocin was synthesized by Katsoyannis and du Vigneaud[2] and kindly supplied by them for pharmacological study in this Department. It is unique among the neurohypophysial hormones and analogues that we have studied in that it has activities on the isolated hen or turtle oviduct, on frog water-balance and diuresis, and on water transport across the bladder-wall of the frog far greater than one would predict from its vasopressor and oxytocic potencies.

Heller[3,4] demonstrated that neurohypophysial extracts from fish, frog, snake and pigeon have frog water-balance activity much greater than that of mammalian extracts. This observation suggests that they contain a peptide active on frog water-balance that is neither oxytocin nor vasopressin. Since arginine vasotocin is such a peptide and has been tentatively identified in the avian neurohypophysis, we have studied neurohypophysial extracts from cold-blooded vertebrates to determine if their pharmacological characteristics are consistent with those of arginine vasotocin.

Extracts from the green turtle (*Chelonia mydas*), the bullfrog (*Rana catesbiana*), the common American toad (*Bufo americanus*), the pollack (*Pollachius virens*), and the marine lamprey (*Petromyzon marinus*) were assayed by rat vasopressor, rat antidiuretic, rat uterus with and without magnesium, rabbit milk-ejection, hen oviduct, and isolated bullfrog bladder assays[1,5,6]. The ratios of potencies by pressor, antidiuretic, hen uterus and frog bladder assays were, in each case, completely consistent with the relative activities of arginine vasotocin on these assays. In all but *Petromyzon* there was more rat uterus and milk-ejection activity than could be due to arginine vasotocin alone. This is consistent with the assumption that oxytocin is also present in all but the lamprey.

One extract of the neurohypophysis of *Squalus acanthias* (spiny dogfish) has also been subjected to this series of assays. Its pharmacological profile is not that of arginine vasotocin, oxytocin, or of any analogue that we have yet studied.

We can advance the hypothesis, therefore, that arginine vasotocin and oxytocin occur in birds, reptiles, amphibians and teleosts. Arginine vasotocin also appears in the most primitive living vertebrates, the cyclostomes. The one elasmobranch studied appears to possess an active peptide unlike any that we have encountered elsewhere among the vertebrates.

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[1] Munsick, R. A., Sawyer, W. H., and van Dyke, H. B., *Fed. Proc.*, **18**, 110 (1959).
[2] Katsoyannis, P. G., and du Vigneaud, V., *J. Biol. Chem.*, **233**, 1352 (1958).
[3] Heller, H., *J. Physiol.*, **100**, 125 (1941).
[4] Heller, H., *J. Physiol.*, **101**, 317 (1942).
[5] van Dyke, H. B., Munsick, R. A., and Sawyer, W. H., *Fed. Proc.*, **18**, 163 (1959).
[6] Sawyer, W. H., Twenty-first Int. Cong. Physiol., Buenos Aires, 1959, Communications, p. 244.

Arginine Vasotocin

IN connexion with the programme being carried out in this Department on the relationship of the structures of the posterior pituitary hormones to their biological properties, an analogue was synthesized[1] containing the ring of oxytocin attached to the side-chain of arginine vasopressin; for convenience, this substance was called arginine vasotocin. This highly purified compound was tested for its uterus-contracting activity with the isolated rat uterus and for its pressor activity in the rat. Our best information at the present time is that the arginine vasotocin has approximately twice the rat-uterus contracting activity and about one-fourth the rat-pressor activity of arginine vasopressin.

Although the arginine vasotocin is not as active with regard to pressor activity as arginine vasopressin, the high pressor activity of the vasotocin is noteworthy in indicating the contribution to pressor activity of the basic amino-acid in the side-chain, since oxytocin, which differs from arginine vasotocin in having leucine in place of arginine in the side-chain, has approximately one-tenth the pressor activity of the arginine vasotocin. The results also indicate that the phenylalanine in the ring of arginine vasopressin is of significance to pressor activity, since the vasopressin is more active than vasotocin, which has *iso*leucine in place of the phenylalanine. Furthermore, the fact that arginine vasotocin has twice the oxytocic activity of arginine vasopressin indicates that *iso*leucine in place of phenylalanine raises the oxytocic activity. In other words, replacement of phenylalanine by *iso*leucine has decreased the pressor activity and raised the oxytocic activity.

Because of the interesting pharmacological properties of arginine vasotocin, a sample of the synthetic product was made available to Prof. H. B. van Dyke for more extensive pharmacological investigation.

In view of the strong evidence of the possible occurrence of arginine vasotocin in non-mammalian sources adduced by Sawyer, Munsick and van Dyke (preceding communication) and the possible correlation of their work with the results of Pickering and Heller (preceding communication) on the occurrence of an active peptide in certain non-mammalian vertebrate neurohypophyses, it was felt that this brief communication on arginine vasotocin would be appropriate.

If subsequent chemical results substantiate the evidence for the occurrence in Nature of arginine vasotocin, the synthesis of the arginine vasotocin would represent a remarkable example of the synthesis of a polypeptide hormone before its identification as a natural product.

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[1] Katsoyannis, P. G., and du Vigneaud, V., *J. Biol. Chem.*, **233**, 1352 (1958).

COUNTER-CURRENT DISTRIBUTION OF PROTEINS IN AQUEOUS POLYMER PHASE SYSTEMS

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THE method of counter-current distribution has been shown to be one of the most efficient tools for analysing mixtures of substances of biochemical interest such as polypeptides. So far, it has mainly been applied to low molecular substances but recently a number of proteins such as insulin[1], adrenocorticotropine[2], growth hormone[2], lactogenic hormone[2], lysozyme[3,4], ribonuclease[4], casein[5–7], serum albumin[8], and hæmoglobin[9] have also been successfully distributed. One of the advantageous features of counter-current distribution is that it is based upon several steps each involving a state of equilibrium between two liquid phases. Generally a state of equilibrium is supposed to be more easily obtained between two liquid phases than between, for example, a solid phase and a liquid phase. For macromolecules this difference in equilibrium behaviour may be expected to be more pronounced than for low molecular weight substances. It would therefore be of the greatest interest if counter-current distribution can be applied also for large molecular weight substances such as proteins and nucleic acids. Many difficulties present themselves, however, when carrying out liquid–liquid extractions of proteins. Thus they show a general tendency, due to their large molecular size, to be distributed unilaterally and there is always a risk of denaturation which may be caused by the interfaces and the organic solvents of the phase system. In some cases, for example, with serum albumin[8], these difficulties have been successfully avoided by introducing complexing agents such as trichloracetic acid. The effect of these is to stabilize the protein against denaturation and to make it more soluble in the organic phase.

The present communication describes another approach to overcome these difficulties, namely, the

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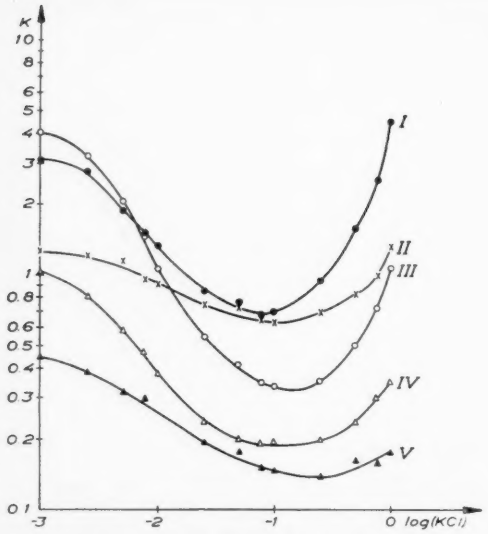

Fig. 1. The partition coefficient (K) as a function of potassium chloride concentration for a number of proteins in a two-phase system of dextran and polyethylene glycol. The curves represent phycocyanin (I), barley albumin (II), phycoerythrin (III), cæruloplasmin (IV), and human serum albumin (V). The system contains 0·005 mole potassium dihydrogen phosphate, 0·005 mole potassium monohydrogen phosphate, and potassium chloride as indicated, all per 976 ml. of the phase system. (976 ml. is the volume of 1 kgm. standard phase system which has a composition of 7 per cent (w/w) dextran, 4·4 per cent (w/w) polyethylene glycol, and 88·6 per cent (w/w) water.) The protein in the two phases was estimated by determining the extinction at 545 mμ for phycoerythrin, the extinction at 620 mμ for phycocyanin, the nitrogen according to the Kjeldahl method for serum albumin, the enzyme activity for cæruloplasmin, and with the biuret method for barley albumin

use of aqueous polymer phase systems[10,11] based upon the fact that different polymer solutions in general are not miscible[12,13]. These systems are obtained by mixing water solutions of two different polymers, for example, dextran and methylcellulose or dextran and polyethylene glycol. Of the two phases in such a system the one phase usually contains most of one polymer and the other phase most of the other polymer. The important point is that the water content is high in both phases and that the interfacial tension is very small. Most proteins will, therefore, dissolve easily without denaturation. This has been demonstrated for a number of proteins[11] and viruses[14] in a dextran–methylcellulose system. The same system has also been used for the enrichment and purification of viruses[15,16] and for the fractionation of brain microsomes[17].

For the present study we have used a system of dextran and polyethylene glycol mainly because its phases have comparatively low viscosities, particularly the top phase (the polyethylene glycol-rich phase), which is the moving one. In addition, reasonable times for phase separation (15–30 min.) may be obtained by this system. The following fractions of the polymers were found to give suitable conditions: dextran with a viscosity number of 0·48 dl./gm. ('Dextran 500' from Pharmacia, Uppsala, Sweden, and 'Carbowax polyethylene glycol 6000' from Carbide and Carbon Chemicals Co., U.S.A.). A phase diagram of the system, and the way in which the polymers may be purified before use, will be described elsewhere[18,19]. The phase system which has been used throughout contained 7 per cent (w/w) dextran and 4·4 per cent (w/w) polyethylene glycol, the rest being water or buffer. The compositions of the upper and lower phases of this system at + 20° C. are 0·2 per cent (w/w) dextran, 7·7 per cent (w/w) polyethylene glycol, 92·1 per cent (w/w) water and 14·4 per cent (w/w) dextran, 0·9 per cent (w/w) polyethylene glycol, 84·7 per cent (w/w) water, respectively. The viscosities of the top phase and the bottom phase are about 4 and 60 centipoises, respectively.

A striking characteristic of this system is the great dependence on the salt composition of the partition coefficient (K) for many proteins. Thus, for example, phycoerythrin is mainly in the top phase ($K = 5$) in the presence of 0·01 M phosphate buffer (pH 6·8). If increasing amounts of potassium chloride are added the protein is pushed down into the bottom phase until a minimum value of $K = 0{\cdot}33$ is obtained. Further addition of potassium chloride then causes the protein to be transferred to the top phase again. Fig. 1 serves to illustrate this salt effect for a number of proteins and it may be seen there that it is rather different for different proteins. This salt effect has been studied systematically and will be described elsewhere[18,19].

The counter-current distributions were run on the two proteins phycoerythrin ($M = 290{,}000$) and phycocyanin ($M = 150{,}000$), which had been isolated from the red alga *Ceramium rubrum*[20] and purified by twice repeated chromatography on calcium phosphate[21]. An automatic apparatus, constructed similarly to the Craig glass apparatus[5], was used. The volumes of the phases were about 10 ml. each, the settling time 35 min., and the number of shakings 50. The results from three experiments are given in Figs. 2, 3 and 4. The aim of the two first experiments (Figs. 2 and 3) was to see if the distribution of the protein followed the theoretical distribution law. A K-value of about 1 was desirable for these experiments and the salt composition necessary to give this was selected with the aid of the curves of Fig. 1. As may be seen in Figs. 2 and 3, the experimental distribution curve fits the theoretical one very

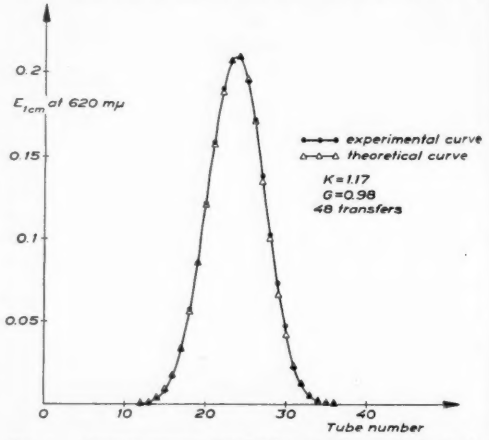

Fig. 2. Counter-current distribution of phycoerythrin in a dextran–polyethylene glycol system containing 0·005 mole potassium monohydrogen phosphate, 0·005 mole potassium dihydrogen phosphate and 0·022 mole potassium chloride per 976 ml. of the phase system. The first tube was charged with 28·2 mgm. protein. Extinction was measured for samples from the bottom phase diluted with equal weight of water. The K value was determined experimentally and the G value (the amount of protein in the top phase divided by the amount of protein in the bottom phase) was calculated from the position of the maximum of the experimental curve

well, indicating that the partition coefficient of the proteins in this phase system is a true Nernst partition coefficient. The salt mixture which would give a large separation factor necessary for the separation of phycoerythrin and phycocyanin could also be selected from the curves of Fig. 1. Thus a salt concentration of 0·01 mole potassium phosphate and 0·75 mole potassium chloride per kgm. phase system was used in the experiment depicted in Fig. 4. The K-value was determined experimentally for each tube. These are plotted in Fig. 4, where it may be seen that while the K-value is fairly constant for phycocyanin it increases with the tube number for phycoerythrin, indicating heterogeneity for the latter protein. Such heterogeneity is also known from chromatography on calcium phosphate[21]. Heterogeneity could not, however, be revealed by precipitation tests according to Ouchterlony[22] since the phycoerythrin of the front and the trailing part of the peak gave identical reactions.

The proteins studied here are coloured and could easily be measured by their strong absorption in the visible spectrum. For uncoloured proteins the estimation of protein concentrations is more tedious. Thus, before measuring the ultra-violet absorption at 280 mμ the phases have to be diluted two to three times and then cleared by high-speed centrifugation for a few minutes. Even then it is sometimes difficult to get reproducible values when a small amount of protein is present. The dextran and polyethylene glycol interfere in the method of protein determination according to Lowry *et al.*[23]. The biuret method[24] works for the polyethylene glycol-rich top phase. Nitrogen determination according to Kjeldahl may be used but this is rather time-consuming when many samples have to be analysed. A simple and rapid modification of these methods for protein estimation in a dextran–polyethylene glycol system is therefore needed.

There is no general method like, for example, dialysis for removing the polymers from the protein. On the other hand, there are so many other possibilities for achieving this purification that for a given protein a suitable method may usually be selected. Two ways in which phycoerythrin was recovered may serve as examples.

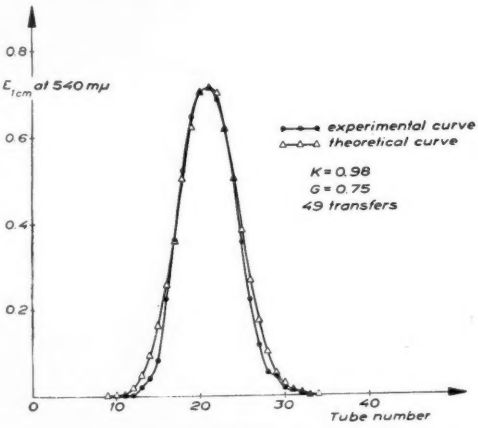

Fig. 3. Counter-current distribution of phycocyanin in the same system as in Fig. 2. The first tube was charged with 7 ml. of a solution of phycocyanin with an extinction of 20·3 when measured at 620 mμ in a 1-cm. cell. The K and G values indicated were obtained as in Fig. 2

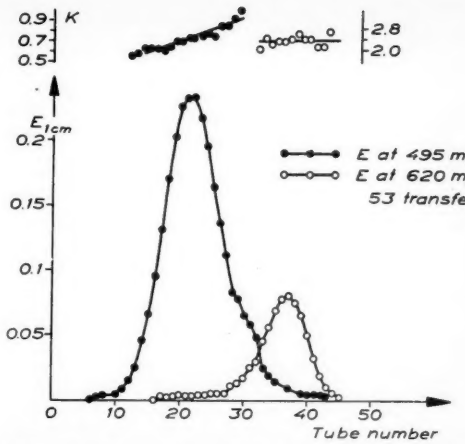

Fig. 4. Counter-current distribution of a mixture of phycoerythrin (filled circles) and phycocyanin (open circles) in a dextran–polyethylene glycol system containing 0·005 mole potassium monohydrogen phosphate, 0·005 mole potassium dihydrogen phosphate, and 0·75 mole potassium chloride per 976 ml. of the phase system. The protein mixture was put into the first two tubes. The extinctions given are those of the top phase. The K values plotted above have been determined experimentally. The estimation of protein in the two phases was done by determination of the extinction at 495 mμ for phycoerythrin and the extinction at 620 mμ for phycocyanin

By the first method the mixture is passed through a column of calcium phosphate at 0·005 M potassium phosphate buffer (pH 6·8). The phycoerythrin is adsorbed while dextran and polyethylene glycol are not and they can therefore be washed away by the buffer. The phycoerythrin is then eluted with 0·1 M phosphate buffer. That the polymers had been washed away completely was checked by the anthrone method[25] for dextran and the method of precipitation of the barium derivative with phosphomolybdic acid for polyethylene glycol[26]. The presence of dextran and polyethylene glycol does not change the absorption capacity of calcium phosphate for phycoerythrin; it only reduces the time of flow.

The second method involves the following procedure: ammonium sulphate is added to 1 : 1 of a mixture containing the phycoerythrin, 3·5 per cent (w/w) dextran, and 2·2 per cent (w/w) polyethylene glycol until a concentration of 1 M is obtained. A top phase of 70 ml. is formed and contains all the phycoerythrin. It is dialysed against a 30 per cent (w/w) solution of polyethylene glycol containing 0·1 M sodium chloride and then added to 17 gm. of a 30 per cent (w/w) solution of a low molecular weight fraction of dextran (viscosity number 0·05). The phycoerythrin is almost entirely transferred to the small bottom phase of this system, which had a volume of 7 ml. and contained no polyethylene glycol. This phase is collected, dialysed, and the protein precipitated by the addition of ammonium sulphate.

All these procedures for the recovery of the protein have the advantage that the protein is concentrated into a small volume, a step which is often desirable prior to further purification or analysis.

We wish to thank Prof. A. Tiselius for his stimulating interest in this work, and Dr. L. Philipsson of the Institute of Hygiene and Bacteriology, Uppsala, for carrying out the immunological tests. The work has been aided financially by grants to the Institute of Biochemistry, Uppsala, from the Swedish Technical Research Council and from the U.S. Department of

the Army through its European Office, under contract number *Da*-91-508-*EUC*-287. One of us (E. J. N.) wishes to thank the Ministry of Education, Belgium, for a grant.

[1] Harfenist, E. J., and Craig, L. C., *J. Amer. Chem. Soc.*, **74**, 3083 (1952).
[2] Li, C. H., "Adv. in Protein Chemistry", **11**, 101 (Academic Press, New York, 1956).
[3] Craenhals, E., and Leonis, J., *Bull. soc. chim. Belges*, **64**, 58 (1955).
[4] King, T. P., and Craig, L. C., *J. Amer. Chem. Soc.*, **80**, 3366 (1958).
[5] Tavel, P. v., and Signer, R., "Adv. in Protein Chemistry", **11**, 237 (Academic Press, New York, 1956).
[6] Walter, J. O., Dissertation, Bern (1952).
[7] Ellfolk, N., *Acta Chem. Scand.*, **11**, 1317 (1957).
[8] Hausmann, W., and Craig, L. C., *J. Amer. Chem. Soc.*, **80**, 2703 (1958).
[9] Hill, R. J., and Craig, L. C., *J. Amer. Chem. Soc.*, **81**, 2272 (1959)
[10] Albertsson, P. Å., *Biochim. Biophys. Acta*, **27**, 378 (1958).
[11] Albertsson, P. Å., *Nature*, **182**, 709 (1958).
[12] Dobry, A., and Boyer-Kawenski, F., *J. Polymer Sci.*, **2**, 90 (1947).
[13] Dobry, A., *Bull. Soc. chim. Belges*, **57**, 280 (1948).
[14] Albertsson, P. Å., and Frick, G., *Biochim. Biophys. Acta* (in the press).
[15] Frick, G., and Albertsson, P. Å., *Nature*, **183**, 1070 (1959).
[16] Wesslén, T., Albertsson, P. Å., and Philipsson, L., *Arch. ges. Virusfors.* (in the press).
[17] Albertsson, P. Å., Hanzon, V., and Toschi, G., *J. Ultrastructure Res.*, **2**, 366 (1959).
[18] Albertsson, P. Å., and Nyns, Ed. J. (in preparation).
[19] Albertsson, P. Å. (in preparation).
[20] Kylin, H., *Z. physiol. Chem.*, **69**, 169 (1910).
[21] Tiselius, A., Hjertén, S., and Levin, Ö., *Arch. Biochem. Biophys.*, **65**, 132 (1956).
[22] Ouchterlony, O., *Acta Pathol. Microbiol. Scand.*, **26**, 507 (1949).
[23] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. G., *J. Biol. Chem.*, **193**, 265 (1951).
[24] Weichselbaum, T. E., *Amer. J. Clin. Path., Tech. Sect.*, **10**, 40 (1946).
[25] Crane, E. W., *Org. Chem. Bull.*, **23**, No. 1 (1951).
[26] Preston, O., *Nature*, **164**, 242 (1949).

PROPERTIES AND INHERITANCE OF HÆMOGLOBIN BY ASYMMETRIC RECOMBINATION

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A MOLECULE of human adult hæmoglobin, which contains two types of polypeptide chains designated the α- and β-chains[1], dissociates into subunits α_2 and β_2 in dilute acid and re-associates to the native molecule $\alpha_2\beta_2$ in neutral solution[2,3]. (A different nomenclature has been used in previous publications (refs. 2, 4 and 5). Identification of the aberrant chain of hæmoglobin *S* (refs. 7 and 8) permits adoption of a uniform nomenclature based on the proposal of Rhinesmith, Schroeder and Martin (ref. 1). A comparative chart of the two systems of nomenclature has been given elsewhere (ref. 3). Recombination of an acid-dissociated mixture of two hæmoglobins that differ from each other in one of the two pairs of chains results in exchange of the sub-unit common to the two forms without formation of new molecular species[3,4]. Recombination of two hæmoglobins that differ from each other in both chains apparently results in formation of new molecular species[5].

The effect of recombining two hæmoglobins that differ in both chains has been observed in the present work with mixtures of human carbonmonoxyhæmoglobin *A* and canine carbonmonoxyhæmoglobin. Acidification of equimolar mixtures of these hæmoglobins to *p*H 4·7 followed by neutralization[4] yielded two new electrophoretic components in addition to the original components (Fig. 1). The absorption spectrum of the recombined mixture is the same as that of an untreated mixture. Canine carbonmonoxyhæmoglobin migrates more slowly than carbonmonoxyhæmoglobin *A* at *p*H 6·8. One of the new components migrates more rapidly than *A* and the other migrates more slowly than the canine form. The relative mobilities of these new components and increase in their proportions with time of exposure to dilute acid (Table 1) are consistent with the occurrence of a relatively slow exchange reaction in which mixed molecules are formed. The net change (formation of new species at the expense of the original components) may be represented by the reaction :

$$\underset{A\ A}{\alpha_2\beta_2} + \underset{\text{can. can.}}{\alpha_2\ \beta_2} = \underset{A\ \text{can.}}{\alpha_2\beta_2} + \underset{\text{can. } A}{\alpha_2\ \beta_2}$$

(The α and β polypeptide chains of human hæmoglobin have been defined according to their N-terminal amino-acid sequences (ref. 1). Since these sequences are not known for canine hæmoglobin, our use of this terminology here depends upon the fact that the chains of canine hæmoglobin appear to be configurational analogues of the α- and β-chains.)

Table 1. Composition of Canine and Human Hæmoglobin Mixtures before and after Recombination

Dissociation time	Electrophoretic composition : per cent of total*			
	New slow	Canine	*A*	New fast
Control	—	41	59	—
20 min.	10	36	44	10
40 min.	11	39	39	11
4 hr.	22	28	32	18
22 hr.	31	24	21	24

* All samples contained 50 per cent each of human and canine hæmoglobin before recombination. Percentages shown are probably anomalous because of low concentration of buffer-ion.

Vinograd, Hutchinson and Schroeder[6] and Ingram[7] have shown that the β-chain is the aberrant chain of hæmoglobin *S*, and Hunt and Ingram[8] have shown that the inherited amino-acid substitutions of hæmoglobins *S* and *C* occur in the identical chain position. Recombination with hæmoglobins *S* and *C* of hæmoglobins *D*, *E* and *J* from families previously reported by one of us[9,10] resulted in no significant change in electrophoretic composition. A component migrating between hæmoglobins *A* and *I* appeared when hæmoglobin *I* from the family reported by Rucknagel, Page and Jensen[11] was recombined with hæmoglobin *S* (Fig. 2). Changes in composition were also observed when Hopkins-2 hæmoglobin[12] was recombined with hæmoglobins *S*, *C* and *J*.

No evidence of recombination was obtained from acidification and neutralization of mixtures of bovine carbonmonoxyhæmoglobin *A* (ref. 13) with human carbonmonoxyhæmoglobin *A*, canine carbonmonoxy-

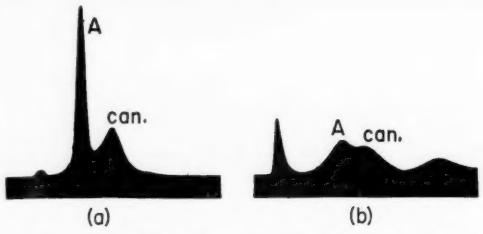

Fig. 1. Ascending limb electrophoretic patterns of 1 per cent carbonmonoxyhæmoglobin in phosphate buffer of *p*H 6·8, Γ/2 0·02. The direction of migration is from right to left. (*a*) *A*, canine control. (*b*) *A*, canine recombined after 4 hr. dissociation at *p*H 4·7

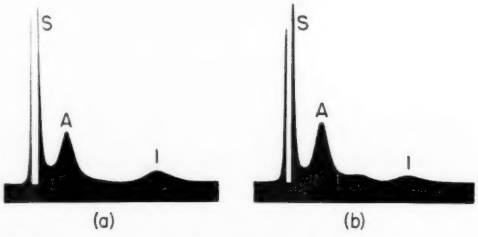

Fig. 2. Ascending limb electrophoretic patterns of 1 per cent carbonmonoxyhæmoglobin in phosphate buffer of *p*H 6·3, Γ/2 0·02. The direction of migration is from right to left. (*a*) *S*, *A*, *I* control. (*b*) *S*, *A*, *I* recombined

hæmoglobin or bovine ferrihæmoglobin *A*. Human and canine carbonmonoxyhæmoglobins, which recombine with each other, also recombine with their respective ferrihæmoglobins to form an intermediate component. The postulated structure of this component, a molecule with two carbonmonoxyhæms and two ferrihæms, is the same as that of an intermediate compound in the oxidation of carbonmonoxyhæmoglobin to ferrihæmoglobin[2,14]. Although it is possible that sub-units of one hæmoglobin would be physically incompatible with those of another[4], it is not likely that sub-units which differ only in the chemical state of the hæms would be incompatible. Therefore the absence of an intermediate component in an acidified and neutralized mixture of the carbonmonoxy- and ferri- forms of bovine hæmoglobin indicates in all probability that the acidified mixture does not contain sub-units, that is, that bovine hæmoglobin, unlike human and canine hæmoglobins, does not dissociate at *p*H 4·7. This conclusion is consistent with other evidence of structural stability of the bovine hæmoglobin molecule, namely, its extremely high resistance to alkali denaturation[15].

These experiments suggest several applications of the recombination method. Reversible dissociability of a hæmoglobin under specified conditions can be tested by subjecting a mixture of its carbonmonoxy- and ferri- forms to these conditions. The altered chain of a dissociable abnormal hæmoglobin can be rapidly identified by electrophoretic analysis after recombination with a previously characterized dissociable form such as hæmoglobin *S*. Change in electrophoretic composition will presumably occur if the abnormal hæmoglobin dissociates reversibly and differs from hæmoglobin *S* in both chains; but no change will occur if it is abnormal only in the β-chain, the abnormal chain of *S*. Although some of our samples have not been tested for dissociability, the results in comparable cases agree with those obtained by Ingram[7] by chemical analyses. The donors of hæmoglobin *I* of our earlier report[5] and the present investigation are not known to be related. Both have mixtures of hæmoglobins *A* and *I* in their red cells; however, the proportions of *I* are different, about 60 and 20 per cent, respectively. The result in each case points to an α-chain defect in hæmoglobin *I*. The occurrence of an exchange reaction between human and canine hæmoglobins shows that these hæmoglobins have similar configurations at the surface of union of their respective sub-units in spite of large differences in amino-acid composition[16]. Isolation and chemical characterization of mixed molecules produced by recombination will permit identification of the chains in canine and other dissociable hæmoglobins that are configurational analogues of the α- and β-chains of hæmoglobin *A*.

According to Smith and Torbert[12], the gene controlling Hopkins-2 hæmoglobin segregates independently of that controlling hæmoglobin *S*. The changes observed in recombined mixtures containing Hopkins-2 hæmoglobin were small because the proportion of this form in the original sample, a mixture of *A* and Hopkins-2, was low. Nevertheless, the results in several experiments were always consistent with abnormality in the α-chain of Hopkins-2 hæmoglobin. Our results to date, together with the familial data of Smith and Torbert, are thus in accord with independent genetic control of the α- and β-chains. The hæmoglobin of double heterozygotes for *S* and Hopkins-2 has only three electrophoretic components, identified as *S*, *A* and Hopkins-2 (ref. 12), although random association of two different α-chain pairs and two different β-chain pairs, each of which is independently synthesized, would yield four molecular species. The fact that *S* and Hopkins-2 have opposite charge abnormalities with respect to hæmoglobin *A* may account for the apparent inconsistency. A molecule composed of abnormal β-chains from *S* and abnormal α-chains from Hopkins-2 would have approximately the same net charge as *A* and would not separate electrophoretically from *A*. The same mechanism has been proposed to explain the presence of only three electrophoretic components in recombined mixtures of hæmoglobins *C* and *I* (ref. 5).

A more detailed report of this work will be published. We are indebted to Dr. Ernest W. Smith for the sample of Hopkins-2 hæmoglobin.

[1] Rhinesmith, H. S., Schroeder, W. A., and Martin, N., *J. Amer. Chem. Soc.*, **80**, 3358 (1958).
[2] Singer, S. J., and Itano, H. A., *Proc. U.S. Nat. Acad. Sci.*, **45**, 174 (1959).
[3] Itano, H. A., Singer, S. J., and Robinson, E., "Ciba Found. Symp. Human Biochemical Genetics" (Churchill, London, 1959; in the press).
[4] Itano, H. A., and Singer, S. J., *Proc. U.S. Nat. Acad. Sci.*, **44**, 522 (1958).
[5] Itano, H. A., and Robinson, E., *Nature*, **183**, 1799 (1959).
[6] Vinograd, J. R., Hutchinson, W. D., and Schroeder, W. R., *J. Amer. Chem. Soc.*, **81**, 3168 (1959).
[7] Ingram, V. M., *Nature*, **183**, 1795 (1959).
[8] Hunt, J. A., and Ingram, V. M., *Nature*, **181**, 1062 (1958).
[9] Sturgeon, P., Itano, H. A., and Bergren, W. R., *Blood*, **10**, 389 (1955).
[10] Thorup, O. A., Itano, H. A., Wheby, M., and Leavell, B. S., *Science*, **123**, 889 (1956).
[11] Rucknagel, D. L., Page, E. B., and Jensen, W. N., *Blood*, **10**, 999 (1955).
[12] Smith, E. W., and Torbert, J. V., *Bull. Johns Hopkins Hosp.*, **101**, 38 (1958).
[13] Bangham, A. D., *Nature*, **179**, 467 (1957).
[14] Itano, H. A., and Robinson, E., *Biochim. Biophys. Acta*, **29**, 545 (1958).
[15] Haurowitz, F., Hardin, R. L., and Dicks, M., *J. Phys. Chem.*, **58**, 103 (1954).
[16] Roche, J., and Mourgue, M., *Trav. Membres Soc. Chim. Biol.*, **23**, 1329 (1941).

FORTHCOMING EVENTS

(Meetings marked with an asterisk • are open to the public)

Monday, December 7

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. B. B. Jacobsen: "Frequency Patterns for Multiple-Radio-Channel Routes".

University of London (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. Max Derruau (University of Clermont-Ferrand): First of three lectures on the "Central Massif of France".• (Further lectures on December 8 and 10.)

University of London (at Queen Mary College, Mile End Road, London, E.1), at 5.30 p.m.—Dr. M. Eigen (Max-Planck-Institut für Physikalische Chemie, Göttingen): "Relaxation Spectra of Fast Chemical Changes in Solution".•

Manchester Literary and Philosophical Society (in the Reynolds Hall, Manchester College of Science and Technology, Sackville Street, Manchester), at 5.45 p.m.—Dr. J. Needham, F.R.S.: "Hydraulic Engineering and Society in Ancient and Medieval China" (Wilde Memorial Lecture).

Society of Instrument Technology (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. G. Pask: "Discontinuous Skill Teaching Machines".

Tuesday, December 8

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, S.W.1), at 9.45 a.m.—Symposium on "Cyclical Activity in Endocrine Systems".

Society of Chemical Industry, Agriculture Group (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Dr. T. H. Rose and Mr. P. H. Brown: "Experiments Comparing a Number of Conditions for the Improvement of Glasshouse Soils"; Mr. J. Webber and Mr. F. W. Shepherd: "Experiments Comparing Bulky Organic Manures for Horticultural Crops"; Dr. F. Haworth: "Fertilizer Responses of some Vegetable Crops".

Institute of Marine Engineers (at the Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. A. Lindén: "Recent Development of the Gotaverken Engine".

University of London (at Queen Mary College, Mile End Road, London, E.1), at 5.30 p.m.—Dr. M. Eigen (Max-Planck-Institut für Physikalische Chemie, Göttingen): "Proton-conducting H-bond Systems and their Analogies to Electronic Semiconductors".•

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. L. G. Lajtha: "Autoradiography in Bone Marrow Studies". (Fourteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.•) (Further lecture on December 10.)

Royal Aeronautical Society (at 4 Hamilton Place, London, W.1), at 7 p.m.—Dr. E. K. Armstrong and Mr. R. E. Stevenson: "Some Practical Aspects of Compressor Blade Vibration".

Wednesday, December 9

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. J. D. N. Nabarro: "Laboratory Investigations in Endocrinology".•

Institute of Physics (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Sir Edward C. Bullard, F.R.S.: "Geological Time".

Society for Psychical Research (at Leighton House, 12 Holland Park Road, London, W.14), at 6.30 p.m.—Dr. Letitia Fairfield: "Children and Witchcraft".

Oil and Colour Chemists' Association, London Section (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. S. H. Bell: "Electron Microscopy and Paint Technology".

Society for Analytical Chemistry, Biological Methods Group (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Annual General Meeting, followed by Dr. H. J. Wallis, Mr. S. S. Kind and Dr. A. S. Curry: "Biological Methods in Forensic Science".

Women's Engineering Society (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7 p.m.—Dr. Elizabeth Laverick: "Radar and Telecommunications Research and Development".

Chemical Society (at Burlington House, Piccadilly, London, W.1), at 7.30 p.m.—Prof. G. H. Cady: "Some Recent Advances in Fluorocarbon Chemistry".

Wednesday, December 9–Friday, December 11

Crop Protection and Pest Control Exhibition (organized by *World Crops*, at the Seymour Hall, Seymour Place, London, W.1).

Thursday, December 10

Institution of Electrical Engineers, Utilization Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. B. Corbyn and Mr. N. L. Potter: "The Characteristics and Protection of Semiconductor Rectifiers".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. Sheila T. Callender: "Iron Absorption".• (Last of the fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Thursday, December 10–Friday, December 11

British Institute of Radiology, incorporated with the Röntgen Society (at Church House, Westminster, London, S.W.1)—Annual Congress and Exhibition.

Royal Society (at Burlington House, Piccadilly, London, W.1), at 9.30 a.m. daily—Discussion on "The Biology of the Southern Cold Temperate Zone" opened by Prof. C. F. A. Pantin, F.R.S.

Friday, December 11

Royal Aeronautical Society (at the Institution of Mechanical Engineers, Birdcage Walk, Westminster, London, S.W.1).—Symposium on "Flight Safety" (All Day Discussion).

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Prof. A. Haddow, F.R.S.: "The Present and Future Relationship of Cancer Research to Medicine".

Institute of Physics (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. J. F. Sayers: "The Ultrasonic Camera—an Alternative Approach to Ultrasonic Testing".

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, W.C.2), at 6.30 p.m.—Sir Willis Jackson, F.R.S.: "The Trends of Electrical Progress and Their Implications".

Royal Aeronautical Society, Agricultural Aviation Group (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. Peter King: "Aerial Operations Planning in the United Kingdom.—Results and Difficulties".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. E. D. Bishop: "The Menace of Vibration in Engineering".

Saturday, December 12

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Philip Hunter-Jones: "The Desert Locust".•

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer in Mathematics in St. Salvator's College—Joint Clerk to the University Court, College Gate, St. Andrews (December 31).

Lecturer (with a special knowledge of, and an interest in, the vertebrates) in Zoology—The Registrar, King's College (University of Durham), Newcastle-upon-Tyne 1 (December 31).

Librarian (graduate, with a recognized library qualification, and preferably experience in a University, College or Technical Library)—The Principal, Coventry Technical College, Butts, Coventry (December 31).

Senior Lecturer (with a degree in chemical engineering or applied and industrial chemistry, or with experience in the practice of chemical engineering in industry and/or in teaching and research) in Chemical Engineering at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, December 31).

Technician (preferably with experience in a laboratory concerned with physiology, biochemistry, or the culture of micro-organisms) in the Botany Department, University of Canterbury, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (December 31).

Senior Lecturer (with main interest and special qualifications in the field of petrology) in Geology at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, January 4).

Lecturer in Veterinary Anatomy at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, February 27).

Head (graduate, with extensive experience of seed production, preferably including practical farming) of the Multiplication Branch—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

Qualified Technician (with the Diploma in Chemical Pathology Technique) at Launceston General Hospital, Tasmania—The Agent General to Tasmania, 457 Strand, London, W.C.2.

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LETTERS TO THE EDITORS

GEOPHYSICS

Geomagnetic Disturbance and Velocity of Slow-Drift Solar Radio Bursts

A POSSIBLE relation between Type II (slow-drift) bursts of solar radio noise and occurrence of geomagnetic disturbance has been suggested by Maxwell, Thompson and Garmire[1,2] and by Roberts[3]. Excitation of plasma oscillations by an outward-moving stream of particles that later reaches the Earth provides a model consistent with radio observations and with the known relations between solar flares and geomagnetic disturbance. According to this model, as the particle stream moves outward, radiation is induced at the plasma frequency (or at the critical frequency) at locations in the solar corona of progressively lower density. Thus the observed rate of decrease of frequency can be related to a velocity, if some model of coronal density is assumed[4]. Further, we might expect that particles with high velocity at great heights would be more likely to escape from the solar atmosphere and to cause geomagnetic disturbance than those decelerated to low velocity at large heights. Hartz[5] has concluded from frequency drifts deduced from observations at single fixed frequencies that such a relation does indeed exist.

We have determined frequency drift-rates from records of the dynamic spectrum analyzer of the Harvard Radio Astronomy Station at Fort Davis, Texas. We are grateful to Drs. Maxwell and Thompson for their hospitality, which made these measures possible, and for their generosity in supplying some of the values. The records that were studied extended from 100 to 580 Mc./s., and covered the period October, 1956–December 1958.

Values of the frequency drift-rate, df/dt were estimated for the leading edge of the burst, often at several different points for a single burst. Each point in Fig. 1 represents a measurement of df/dt. A burst without observed harmonic structure was assumed to represent the second harmonic; thus both df/dt and the frequency, f, were divided by two before the point was plotted in Fig. 1.

We have looked for a systematic difference in frequency drift-rate between two groups of Type II bursts: those followed by geomagnetic disturbance, and those followed by no disturbance. A burst was considered to be followed by disturbance if, in the interval 24–72 hr. after the burst, there occurred either a value of the daily geomagnetic index $A_p \geq 30$, or a period of 9 hr. for which the sum of the three successive 3-hr. K_p values was ≥ 17. The mean value of A_p for the two days following the burst is 38 for the first group and 10 for the second. Fig. 1*a* represents the relation of df/dt to f for 28 bursts followed by geomagnetic disturbance; Fig. 1*b*, for 20 bursts followed by no disturbance.

The straight lines in each case represent the linear least squares solution for the data. Bursts followed by disturbance determine a line with slope significantly smaller than that for bursts without disturbance. According to Student's t-test, the probability is only 0·005 that the two slopes could arise from data selected at random from a single universe[6]. Although the statistical test takes into account the variance in the measured values, it does not take into account the scarcity of points at high frequencies in Fig. 1*b*, so that this value should be interpreted with caution.

If one interprets the slope $d/df\ (df/dt)$ as a change in velocity with height, the difference between slopes indicates that the positive acceleration of the group of bursts followed by disturbance is significantly larger than the corresponding acceleration for bursts followed by no disturbance. Thus the difference in rate of change of frequency drift-rate for the two groups of bursts is consistent with the identification of the radio source with a stream of particles which escapes from the sun in one case, and fails to escape in the other.

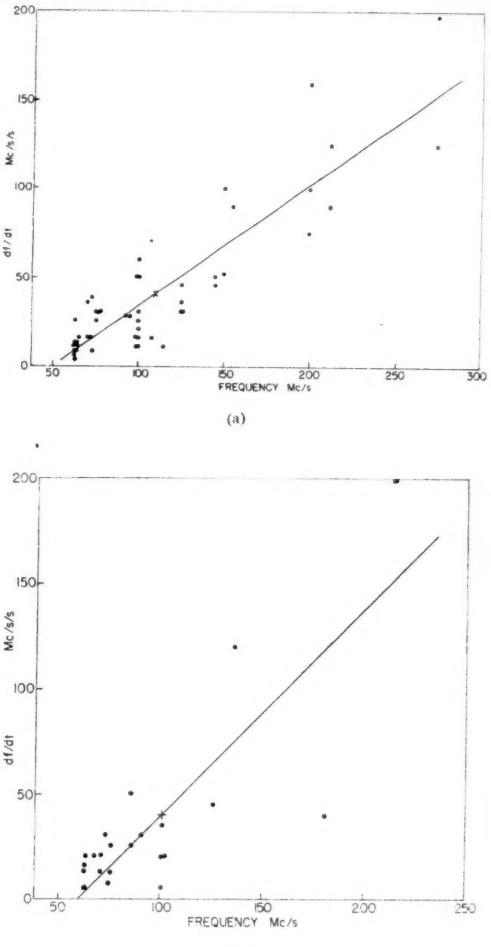

Fig. 1. Frequency drift-rate (ordinate) vs. frequency (abscissa). *a*, for Type II bursts followed by geomagnetic disturbance; *b*, for Type II bursts followed by no disturbance

We have not been successful in interpreting drift-rates of individual bursts in terms of accelerations because of the scarcity and the uncertainty of individual measurements, and because the velocity calculations are very sensitively dependent on the coronal density model assumed. A significant result was obtained only when all of the available measures were combined.

We conclude, then, that frequency drift-rates of Type II bursts, determined from radio spectral observations, indicate a systematically greater acceleration for the sources of bursts followed by geomagnetic disturbance than for the sources of bursts followed by no disturbance.

M. B. Wood
C. S. Warwick

National Bureau of Standards,
Boulder, Colorado.
Oct. 8.

[1] Thompson, A. R., Paris Symposium on Radio Astronomy, 210, R. N. Bracewell, Ed. (Stanford, 1959).
[2] Maxwell, A., Thompson, A. R., and Garmire, G. (to be published).
[3] Roberts, J. S., Paris Symposium on Radio Astronomy, 194, R. N. Bracewell, Ed. (Stanford, 1959).
[4] Wild, J. P., *Aust. J. Sci. Res.*, A, 399 (1950).
[5] Hartz, T. R., Paris Symposium on Radio Astronomy, 554, R. N. Bracewell, Ed. (Stanford, 1959).
[6] Crow, E., Davis, F., and Maxfield, M., Nav. Ord. Report, 3369 (1955).

Natural Remanent Magnetization of Pre-Cambrian Gneiss of Ongul Islands in the Antarctic

A number of rock samples of Pre-Cambrian biotite hornblende granodioritic gneiss from the Ongul Islands, where the Japanese Antarctic Research Base Syowa was set up, were collected by one of us (T.N.) in February 1957 and in January 1959. This was for the purpose of determining magnetic polarization of the antarctic rocks in view of recent progress in palæomagnetism, including the problems of polar wandering and continental drift.

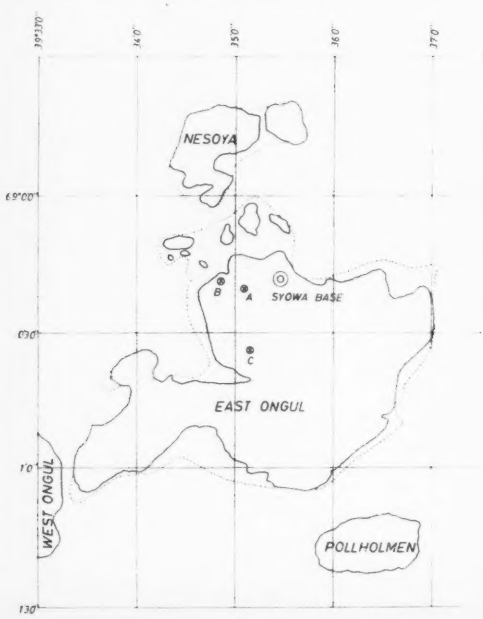

Fig. 1. Localities *A*, *B*, and *C* of sampling Pre-Cambrian gneiss in Ongul Island

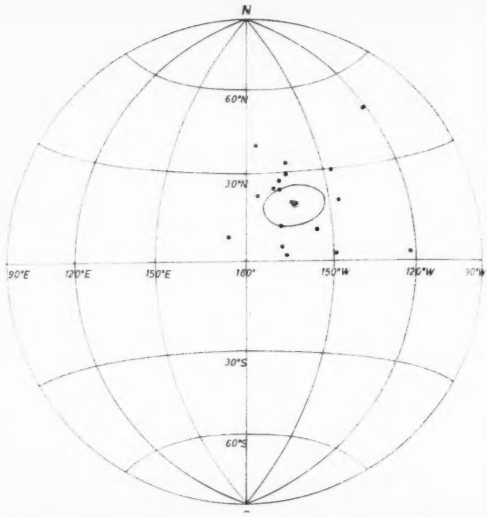

Fig. 2. Position of the north pole of the Earth's centred dipole estimated from magnetic polarization of Antarctic Pre-Cambrian gneiss

The locality of Syowa Base is about 69°00′S. and 39°35′E. Rock samples examined here were collected from three sites in East Ongul Island, as shown in Fig. 1.

These metamorphic rocks *in situ* have a clear banded structure, dip and strike of the gneissosity-planes ranging from 51° to 54° and from N. 20°W. to N. 01°W. respectively.

According to the results of laboratory examination of their magnetic properties, ferromagnetic minerals contained in these rocks are nearly pure magnetites and hematites, having their Curie point at 580°C. and 680°C. respectively, but those responsible for natural remanent magnetization are mainly hematites.

Among a number of collected rock-samples, those which have unstable remanent magnetization are eliminated according to the well known experimental criteria[1] (that is, stability test against d.c. and a.c. magnetic fields and thermal demagnetization).

The results of this test showed that, generally speaking, remanent magnetization of these rocks, intensity of which is very weak compared with the others and is of the order of magnitude of their isothermal remanence, is unstable.

The number of rock specimens whose magnetic polarization was measured was 4 from site *A*, 8 from site *B* and 6 from site *C* (Fig. 1). Intensity and direction of remanent magnetization of these 18 specimens are given in Table 1, and position of the North pole of the earth's centred dipole corresponding to each magnetic polarization is plotted in Fig. 2. The average position of the North pole thus estimated is given as :

lat. = 19°N. long. = 163°W.,

the radius of circle of confidence of 5 per cent being 7°.

At present, the process of formation of the geological structure concerned has not yet been made clear. According to the preliminary results of geological and geomorphological studies of the Ongul Islands[2], however, it seems that the inclination of the planes of banding of gneiss was caused by a certain crustal deformation after completion of metamorphism of those rocks concerned.

Assuming therefore that the gneissosity-planes were horizontal when thermal metamorphism of this

TABLE 1

Sample No.	Intensity (e.m.u./gm. $\times 10^6$)	Direction		Corrected Direction	
		Decli.	Incli.	Decli.	Incli.
*A*1	7·2	N.33°E.	78°	N.73°E.	31°
*A*2	5·8	N.20 E.	40	N.41 E.	8
*A*3	7·6	N.16 W.	50	N.31 E.	30
*A*4	7·4	N. 8 W.	60	N.44 E.	30
Pole position derived from mean values of the above four. lat. 18°N, long. = 137°.5W. Corrected. lat. = 2°.5S, lat. = 96°W.					
*A*1	12·2	N.34°W.	69°	N.52°E.	32°
*A*2	18·6	N.10 W.	68	N.52 E.	33
*A*3	9·0	N.37 W.	61	N.36 E.	40
*A*4	14·4	N.49 W.	40	N. 2 E.	44
*A*5	20·4	N. 9 W.	41	N.25 E.	22
*A*6	22·2	N.42 W.	58	N.38 E.	46
*A*7	13·0	N.29 W.	58	N.33 E.	40
*A*8	21·2	N.33 W.	66	N.46 E.	42
Pole position derived from mean values of the above eight. lat. = 20°.5N, long. = 166°.5W. Corrected. lat. = 3°.5N, long. = 107°.5W.					
*C*1	5·8	N.27°W.	39°	N.10°E.	27°
*C*2	6·8	N.29 W.	42	N.14 E.	30
*C*3	9·2	N.35 W.	64	N.39 E.	42
*C*4	8·6	N.50 W.	70	N.47 E.	48
*C*5	33·6	N.35 W.	62	N.45 E.	43
*C*6	39·2	N.31 W.	50	N.29 E.	40
Pole position derived from mean values of the above six. lat. = 16°N, long. = 168°W. Corrected. lat. = 2°.5N, long. = 115°.5W					

area took place and consequently thermo-remanent magnetization of those rocks was produced, the direction of magnetic polarization of each specimen is transformed to that referred to the assumed ancient co-ordinates of the horizon and the meridian. The direction thus transformed of each specimen is shown also in Table 1.

The average position of the North pole of the earth's dipole corresponding to the transformed magnetic polarization is given as :

$$\text{lat.} = 3°\text{N. and long.} = 107°\text{W.},$$

where the radius of circle of confidence of 5 per cent is 7°.

It may be concluded from this result that the North pole of the Earth's magnetic dipole was situated approximately at 107°W. in longitude near the equator in the Pre-Cambrian, assuming that there has been no remarkable drift and rotation of the antarctic continent. In the southern hemisphere, palæomagnetic studies on Pre-Cambrian rocks in Australia[3] and South Africa[4] have already been carried out. For comparison, the results of these Australian and South African data are shown in Table 2 together with the present one.

Comparing these results to each other, it will be seen that the pole position derived from the present data is in rather good agreement with that derived from the South African data, while the Australian data, especially the last two results in Table 2, are appreciably different from the upper two. It might be suggested that the rough agreement between the present and the South African results can be attributed to the condition that these two localities are situated at nearly the same longitude though they are separated from each other by about 35° in latitude.

TABLE 2

Locality	Author	Pole Position	
		Lat.	Long.
Antarctic	Nagata & Shimizu	3°N.	107°W.
South Africa	Gough (ref. 3)	8°S.	137°W.
Australia	Irving & Green (ref. 3)	30°N.	121°W.
		51°N.	18°W.
		6°N.	14°W.

As for the Pre-Cambrian pole position, a number of data have also been reported with respect to European and American rocks in the northern hemisphere. Although these northern hemisphere data[5] do not give an unique pole position of the earth's dipole, it may be said that most of those pole positions are situated between the equator and 35°N.

Summarizing all data obtained hitherto, including the present antarctic one, it may be substantially said that the pole-position of the earth's dipole, estimated by the palæomagnetic method, was situated near the equator in the Pre-Cambrian Period.

T. NAGATA
Y. SHIMIZU

Geophysical Institute,
University of Tokyo.
Aug. 27.

[1] Nagata, T., 'Special Report on Palæomagnetism', Trans. Rome Meeting A.G.A., I.U.G.G., 343.
[2] Yoshikawa, T., and Toya, H., Antarctic Report, No. 1, 1 (1957). Tatsumi, T., and Kikuchi, T., Antarctic Report, No. 7 (1959) (in the press).
[3] Gough, D. I., *Mon. Not. Roy. Astro. Soc., Geophys. Suppl.*, **7**, 196 (1956).
[4] Irving, E., and Green, R., *Geophys. J. Roy. Astro. Soc.*, **1**, 64 (1958).
[5] See, for example, a summary by Irving, E., *Geophys. J. Roy. Astro. Soc.*, **2**, 51 (1959).

GEOLOGY

The Geological Time-Scale

SINCE my communication on this subject was written[1], much has happened. Prof. J. L. Kulp[2] has just published a time-scale in which the duration of the geological periods is given as follows : Ordovician, 490–430 m.y. ; Silurian 430–410 m.y. ; Devonian 410–355 m.y. ; Mississippian 355–330 m.y. ; Pennsylvanian 330–275 m.y. ; Permian 275–220 m.y. ; Triassic 220–180 m.y. ; Jurassic 180–135 m.y. ; Cretaceous 135–70 m.y. An almost identical geochronology ($\pm$5–10 m.y.), awaiting publication, was communicated to me privately by Prof. A. Holmes[3] a month ago. The Oxford age of 305 m.y. for Dartmoor granite (by potassium/argon assay on biotite) has been confirmed by Dr. H. Faul[4] of U.S. Geological Survey (292 m.y. and 288 m.y. on the same rock by the same method). A full isotopic assay of Hercynian pitchblende from Geevor, Cornwall, has yielded to Dr. A. G. Darnley[5] of the Geological Survey of Great Britain a comparable date of 288 m.y. From all this it is confirmed that an extension of the Holmes *B* time-scale is called for, and that the best values are, as one would expect, a little higher than the averages of the experimental results published previously[1]. There is however, no support from recent researches for the conclusion that the Lower Cambrian is older than 600 m.y.

C. F. DAVIDSON

Department of Geology,
University of St. Andrews,
Scotland.
Nov. 4.

[1] *Nature*, **184**, 1310 (Oct. 24, 1959).
[2] Kulp, J. L., Program 1959 Annual Meeting, Geol. Soc. America (Nov. 2, 1959).
[3] Holmes, A. (personal communication, Oct. 6, 1959).
[4] Faul, H. (personal communication, Oct. 1959).
[5] Darnley, A. G., Geol. Surv. Great Britain, Age Determination Rep., 6 (Aug., 1959).

PHYSICS

Bubble Counting on Low-Energy Particle Tracks

The possibility of distinguishing between the tracks of mesons and protons in bubble chambers by measurements of linear bubble density has been investigated.

In the course of an investigation of photomeson and photoproton production from an external target, irradiated by 340 MeV. bremsstralung, using a bubble chamber as particle detector, identification was often provided by inspection of the track ending. Thus a π^+ meson track commonly terminates with a decay $\mu^+ \rightarrow e^+$, and a π^- meson with a disintegration star. Ambiguity exists, however, between π^- meson tracks which have no visible star products and proton tracks. It may be possible to overcome this ambiguity by an examination of multiple coulomb scattering; but this method applies only if the track is at least 2 cm. long, and even then is not always reliable. An investigation was therefore made of the possibility of using bubble-counting methods to aid this identification.

To count bubbles the film was examined under a microscope. The number of bubbles over a range of 0·1 gm./cm.^{2} was counted at a measured distance back from the ending of a track, and this number was converted to bubbles per cm. The bubble density as a function of the mean range is shown in Fig. 1.

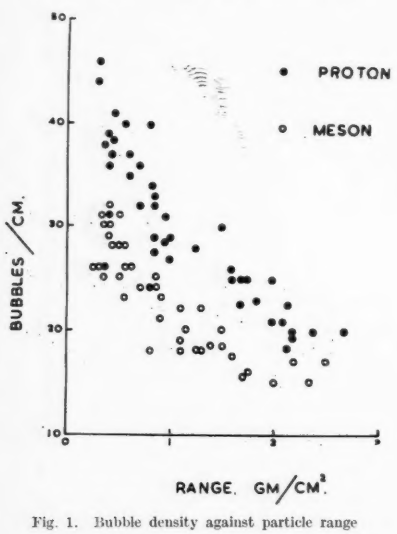

Fig. 1. Bubble density against particle range

Points indicated as circles represent meson tracks identified by their end products. Points indicated by full dots represent tracks showing no such end products. Some of these latter tracks have not been included in this graph since they were long enough to be identified as mesons by examination of the scattering. The separation of mesons and protons into two groups is very striking, the few exceptions being reasonably attributed to mesons which had not been identified as such by scattering considerations.

Within the rather inadequate statistics, the relative numbers of mesons and protons conform to the numbers expected from cross-section calculations.

It appears from these results that bubble counting provides a satisfactory method for the separate identification of protons and mesons.

Since it is believed that δ-rays or secondary electrons are in some way responsible for the creation of bubbles, graphs of bubble density against $1/\beta^2$ have been constructed, notably by Glaser[1] and Blinov[2] and a linear relationship found.

In the present experiment, the energy of the particle of range R was obtained from tables, and from this $1/\beta^2$ was calculated. Graphs of bubble density against $1/\beta^2$ constructed from the results shown in Fig. 1 are shown in Fig. 2.

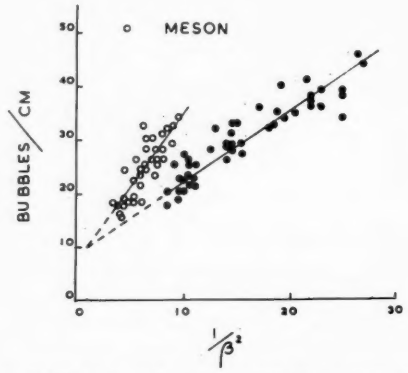

Fig. 2. Bubble density against $1/\beta^2$ ($\beta = V/C$; V = particle velocity)

A straight line is obtained for both mesons and protons, agreeing with the earlier results of Glaser and Blinov. It can be seen, however, that the slope of the curves depends on the mass of the particle, a result not readily explained by current theories. Both curves extrapolate to a value at $1/\beta^2 = 1$ which is identical with the bubble density found on minimum ionizing electron tracks.

Further experiments are being performed to investigate these effects more thoroughly.

I. R. Birss

Department of Natural Philosophy,
The University,
Glasgow, W.2.
Sept. 17.

[1] Glaser, Proc. C.E.R.N. Conference (1956).
[2] Blinov, *J.E.T.P.*, **31**, 762 (1956).

Electron Microscopy of Autoradiographed Radioactive Particles

The combination of the techniques of electron microscopy and autoradiography was first described by Liquier-Milward[1]. O'Brien and one of us (L.A.G.) later described the application of a similar technique to ultra-thin sections of yeast[2]. This communication describes a further modification of the technique for the identification of radioactive particles collected on millipore filters. This work was in support of an investigation into the effects of the inhalation of radioactive particles.

In this procedure, millipore filters, which are an integral part of chambers used to expose lungs of animals to radioactive dusts, are collected and processed for examination with the electron microscope by the method described by Kalmus[3]. Small areas of approximately 2 mm.^{2} are cut out of the central portion of the millipore filter (type *AA* ; plain,

Lovell Chemical Co., Watertown, Mass. 47 mm. diameter) and placed with the collecting-side down on the surface of a formvar- and carbon-coated electron microscope grid. The grids are supported in a Petri dish by a medium-pore sintered-glass filter stick instead of a stainless steel screen platform covered with filter paper, as described by Kalmus. The filter solvent of choice (acetone in this case) is then carefully poured into the Petri dish until the level is about 1 mm. below the surface of the filter stick[4]. Five changes of acetone of 30 minutes duration each are used to accomplish complete dissolution of the filter. It is not essential completely to dissolve the filter but consistently better results are obtained when the filters are dissolved and the grids then processed for autoradiography.

After dissolution of the millipore filter, the grids are air dried, examined with the electron microscope and selected areas photographed. The grids are then secured for application of the emulsion to one end of a small glass peg by means of double-coated scotch tape. The pegs, with the grids in an upright position, are then inserted into holes of slightly larger diameter drilled into a block of stainless steel. The depth of the drilled holes is equal to about one-half the length of the glass pegs. All subsequent steps in the process of autoradiography are carried out under light filtered through a Wratten, series *OA*, filter. Alpha-sensitive Eastman Kodak *NTA* emulsion in gel form (supplied through the courtesy of Eastman Kodak Company, Rochester, N.Y.) is heated at 45°C. until fluid, and then diluted 1:2 with distilled water. The specimen grids are coated by means of a fine wire loop which is dipped into the emulsion and then slowly withdrawn leaving a thin layer of the diluted emulsion in the loop. The emulsion is then placed on the specimen grid by passing the loop down over the stationary grid.

After coating, the grids are placed in a light-tight box and exposed for a suitable length of time at 4 C. With plutonium-239, the radioelement used in this study, three days of exposure was found to be sufficient.

The autoradiograms are then developed for five minutes in Eastman Kodak *D*-19 developer, rinsed twice in tap water, fixed in acid fixer for 15 minutes and thoroughly rinsed in running tap water for 30 minutes. After drying they are examined with an *RCA EMU-2C* electron microscope.

Examples of the results obtained are shown in Figs. 1*a*, 1*b*, and 2. Fig. 1*a* shows a particle of plutonium photographed before application of the emulsion and Fig. 1*b* the same particle after autoradiographic processing of the grid. Fig. 2 illustrates the differentiation of a radioactive from an adjacent non-radioactive particle. Differentiation leading to a more complete knowledge about the physical characteristics of the inhaled radioactive particles is afforded through the application of the methods described in this communication.

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[1] Liquier-Milward, J., *Nature*, **177**, 619 (1956).
[2] O'Brien, R. T., and George, L. A., II, *Nature*, **183**, 1461 (1959).
[3] Kalmus, E. H., *J. App. Phys.*, **25**, 87 (1954).
[4] Mastel, B. (personal communication).

Insulating Particles on Cathode Surfaces: their Effect upon Electrical Discharges

Previous work[1,2] has shown that arcs on the negative electrode of a spark gap, were initiated around poorly conducting inclusions. As a logical development of this finding it became of some importance to differentiate between the respective parts played by inclusions and the metal, from which the cathodes were made, in the initiation of arcs.

Using an experimental procedure and under conditions which have been described[2], inclusion-free samples of copper, platinum and tungsten were used as cathodes in a spark gap, subsequent to metallographic preparation. In this condition arcing did not occur with the maximum available potential difference of 500 volts across the spark gap. The current density at the cathode was about 10^{-4} amp. cm.$^{-2}$

Small quantities of various powders (approximately spherical particle shape) were then placed upon the horizontal surface of the cathodes and the minimum potential across the spark gap necessary to cause arcing of a given particle size was noted.

In these experiments the powders used were of alumina, diamond and niobium carbide. Results are shown in Fig. 1 for copper and platinum cathodes;

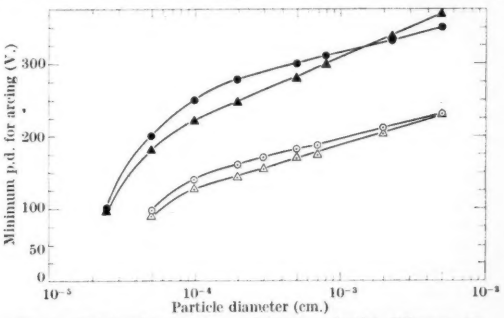

Fig. 1. Relationship between minimum potential difference for arcing and particle size. ●—●, alumina on copper; ▲—▲, alumina on platinum; ○—○, diamond on copper; △—△, diamond on platinum

that for tungsten is omitted for reasons of clarity. Niobium carbide (a good conductor) did not cause arcing, thus refuting the possibility that arcing is due merely to points upon the cathode surface. Microscopical examination subsequent to testing showed that local surface-melting of the cathode had occurred around alumina and diamond particles as observed previously around inclusions.

As can be seen from Fig. 1, there is a small but definite increase in minimum potential difference necessary to cause arcing with

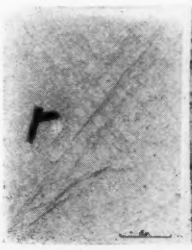

Fig. 1*a*

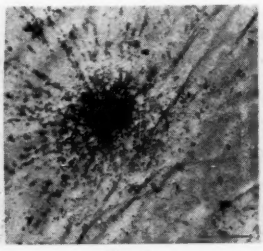

Fig. 1*b*
Scales : 5μ

Fig. 2

increasing particle size. The size determinations (made with an optical microscope and a calibrated micrometer eyepiece) are accurate to ± 5 per cent at sizes above 5×10^{-4} cm. but below 10^{-4} cm. the error increases due to diffraction effects, and over-estimations are very probable. This may in part explain the apparent change in shape of the curves at sizes of about 10^{-4} cm. At even lower particle sizes changes in resistivity will occur and one would therefore predict changes in the shape of the curves at sizes of about 5×10^{-6} cm. The small but definite increase of the potential required for arc initiation with increase of particle size is partly to be attributed to distortion of the electric field as the particles on the cathode acquire an appreciable positive potential with respect to it.

Of special interest is the close proximity of the curves in Fig. 1 for a given powder, indicating virtually no dependence upon the metal involved (curves for tungsten cathode were similar to those shown in Fig. 1), and it therefore appears that arc initiation is dependent purely upon the electrical properties, probably the electrical strength, of poorly-conducting inclusions and surface dusts if these are present. However, the actual quantity of metal evaporated is dependent upon the thermal and electrical properties of the metal.

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[1] Pfeil, P. C. L., and Griffiths, L. B., *Nature*, **183**, 1481 (1959).
[2] Pfeil, P. C. L., and Griffiths, L. B., *J. Nuclear Materials*, **1**, (3) (1959).

Stochastic Rectification of Non-linear Clocks

Sir John Carroll[1] has recently discussed the possibility of recovering an absolute scale of time from the observation of nuclear decay processes. The essence of the following remarks is that the owner of a non-linear clock can rectify it if he is allowed to observe the motion of a Brownian particle (in the sense of Norbert Wiener).

We define a *clock* A to be a deterministic mechanism which supplies at time t a pointer-reading $\alpha(t)$, called *apparent* time, where $\alpha(t)$ as a function of the real variable t is strictly monotonic increasing (it need not be continuous). The clock A will be called *linear* if $\alpha(t)=\lambda+\mu t$ for all t, where λ and μ are (perhaps unknown) constants. The determination of λ and μ for a linear clock is just a matter of the choice of origin and unit of time, and is not under discussion here. Now suppose that an observer possesses a clock A which, for all he knows, may be non-linear, and that he is allowed to employ it in recording the history of a particle which is known by the observer to be describing a one-dimensional Wiener motion. This is to say[2] that, if x_t is the co-ordinate of the particle at true time t, then $x_s - x_t$ (where $s>t$) has a Gaussian distribution with mean value zero and with standard deviation $\sqrt{(s-t)}$, and that the increments x_s-x_t, x_u-x_v, . . . are statistically independent whenever the corresponding intervals $(t, s]$, $(v, u]$, . . . are non-overlapping. Then the observer can construct (retrospectively) a perfect linear clock in the following way.

Let y_α be the value of x_t when $\alpha(t)=\alpha$ (if the value α falls within a 'jump' of the clock-function, then we take t to be the true time $\theta(\alpha)$ at which that jump took place, that is, we complete the 'graph' of the clock-function by drawing vertical lines at all the jumps). Suppose the observer wants to determine the interval T of true time corresponding to the apparent time-interval (0,1). He divides the apparent time-interval from $\alpha=0$ to $\alpha=1$ into 2^n equal sub-intervals of which the r th, say, is (α_{r-1}, α_r) $(r=1,2, \ldots, 2^n=N)$, and observes for each r the position $x(r)$ of the particle (this will be the value of y_α when $\alpha=\alpha_r$); he then computes the sum:

$$S_n = \sum_{r=1}^{N} \left\{ x(r) - x(r-1) \right\}^2$$

Finally a theorem of P. Lévy[3] assures him that, with probability one, $S_n \to T$ when $n \to \infty$.

The proof is very simple; if $t_r=\theta(\alpha_r)$, then the points $t_1, t_2, \ldots$ must lie densely in the true time-interval $(0,T)$, and the theorem of Lévy then gives the result at once.

Any feeling that there is a paradox here can be dissipated as follows; a temporal uniformity is built into the Wiener motion, and it is the statistical independence of the successive increments of x_t which enables the observer to extract this uniformity from the history of a single particle. It would be interesting to characterize those stochastic processes which permit the reconstruction of the time-scale in this or some similar way.

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Oct. 19.

[1] *Nature*, **184**, 260 (1959).
[2] See, for example, Paley, R. E. A. C., and Wiener, N., 'Fourier Transforms in the Complex Domain' (New York, 1934), chapter 9. A distinction must of course be drawn between this mathematical construction and the path of an *actual* Brownian particle.
[3] Lévy, P., *Amer. J. Math.*, **62**, 487 (1940).

Structural Defects in Fused and Crystalline Silica

Samples of fused silica (optical quality from General Electric Co.) in the form of disks about 4 cm. diameter and 0·64 cm. thick were treated with a modification of an etchant material previously used for various types of soda-lime-silica glasses[1]. In the modified solution the sodium ion was replaced by potassium. The composition of the etchant was 2·0 gm. potassium fluoride and 1·2 c.c. hydrochloric acid per 100 c.c. water. The flat surfaces of the disks were polished, and in initial etching treatments, it was observed that scratches and polishing defects were made visible. There was no evidence of flaw patterns on the polished surfaces characteristic of those found in soda-lime-silica glasses. In order to eliminate the effect of the polishing and surface treatments, the disks were split open and the fresh surfaces etched; the optimum time was about 24 hr.

The samples were broken by scratching with a diamond on one surface and tapping on the opposite side until the sample split open with a rapid break. The broken halves were placed immediately in the etchant.

A microscopic examination of these etched surfaces disclosed minute line patterns which in most cases matched exactly on the opposite fracture surfaces. Examples of typical etched surfaces on fused silica are shown in Fig. 1, *A* and *B*. The defects are the contrasting light lines extending through the fields. It may be seen that at their junction points they do not

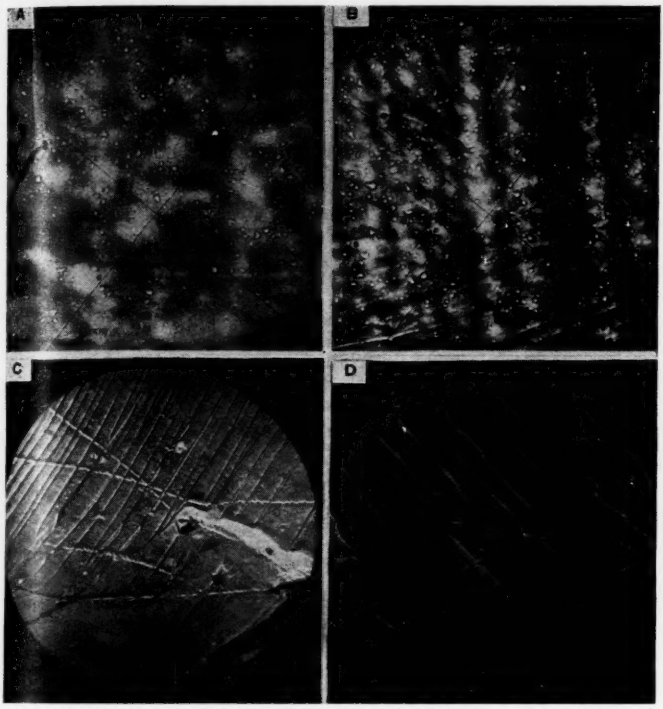

Fig. 1. *A* and *B*, Etch patterns on matching fracture surfaces in fused silica (× *c.* 30); *C* and *D*, Flaws on matching cleavage surfaces in a quartz crystal (× *c.* 56)

appear to cross. This non-crossing effect is typical of flaw patterns found in soda-lime glasses.

There are, however, variations in these etch patterns which are significant and seem to point out basic differences in the flaw types. The lines show up light against the background when the focal plane of the microscope is slightly above the surface of the sample. Under these same conditions, dark contrasting flaw lines were found in soda-lime glasses. The significance of this is that the lines in the silica, instead of being furrows, are actually ridges with respect to the surrounding plane surface. This indicates that these lines are of lower energy with respect to the etchant solution than the surrounding matrix. The fact that these flaw patterns match even though they are located on a dynamically produced crack front suggests that these low-energy etch regions may be microcrystalline planes within the body of the fused silica which were severed by the fracture. In a very few cases, minute flaw lines were formed which did not exactly match on the opposite crack surface; this is evidence of bond rupture produced on the fracture plane by the breakage stresses.

An application of this same etching solution to cleavage surfaces on quartz crystals did not show the same effect as observed with the fused silica. It was necessary to use an etching time of 5–6 days in order to obtain a pattern; and even in this case, no definite matching lines were observed on the treated surfaces. It appeared that the etchant did not act in as selective a manner as on the fused silica. The etchant solution was, therefore, further modified by adding the dichromate ion in the form of 0·2 weight per cent $K_2Cr_2O_7$. It is well known that chromium ions adsorb on glass surfaces; in fact, a chromate-containing mixture is used as a cleaning solution for glassware. Butler and Johnston[2] estimated that a 0·03 monolayer of chromium is retained by a cleaned fused silica surface after rinsing and drying. It was thought, therefore, that the chromium might adsorb preferentially and give the etchant solution a more selective action on quartz.

The quartz crystals used were from Hot Springs, Arkansas (supplied by Wards Natural Science Establishment); about 5 cm. long and 2 cm. in diameter. The fresh cleavage surfaces were produced by impacting the crystal with a concentrated force.

It was observed that the matching flaw patterns were developed in quartz after approximately thirty days in the solution. Opposite sides of a cleavage surface are shown in Fig. 1, *C* and *D*. The photomicrographs are of polystyrene replicas[3] stripped from the etched surfaces. The cleavage plane shown was approximately parallel to the basal plane of the crystal. The diagonal lines in the photomicrographs are the usual rib marks formed by fracture under impact stress. The matching flaws are the sharp etch lines extending through the rib marks. In some cases, the lines appear to meet one another but do not cross. An example of crossing lines is shown in the upper region of the fields. There are also two etch crystals near the bottom of both photomicrographs which appear to originate from the same sources. These matching pits may be the ends of defect lines normal to the cleavage plane; possibly minute growth centres of the type shown by Zimonyi[4].

The patterns found in quartz appear to represent a very positive slip mechanism in the silica network. Willis[5] has shown a slip plane 3,000 A. high on the major rhombohedral face of quartz. These slip planes do not disclose the resealing and movement effects found in flaw patterns on the fracture surfaces of soda-lime-silica glasses. The slip process may initiate along planes of weakened or broken silicon-oxygen bonds.

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[1] Levengood, W. C., *J. App. Phys.*, **30**, 378 (1959).
[2] Butler, E. B., and Johnston, W. H., *Science*, **120**, 543 (1954).
[3] Geilmann, W., and Tolg, G., *Glastech. Ber.*, **28**, 299 (1955).
[4] Zimonyi, Gy, *Acta Phys. Acad. Sci. Hung.*, **8**, 119 (1957).
[5] Willis, B. T. M., *Nature*, **170**, 115 (1952).

A Molecular Approach to the Problem of Viscoelasticity

In a previous communication[1], one of us made a tentative attempt to interpret the rheological behaviour of hydrogen-bonded solids in terms of their molecular and thermodynamic parameters. This theory was elaborated in a later publication[2], in which evidence from a number of sources was adduced for its validity. Further evidence was obtained from other sources showing that the theory was acceptable[3,4]. However, the evidence presented

in these papers was indirect. We wish to report here a more direct confirmation of the major hypothesis made in this theory.

The basic hypothesis is that the mechanical properties of such materials as paper, regenerated cellulose sheets and fibres, and ice are controlled by the number, n, of hydrogen bonds per unit volume of material subjected to strain, and by the potential energy, U_0, of these bonds. Thus the theory predicts that Young's modulus, E, for such materials is directly proportional to $n^{\frac{1}{3}}$. The following series of experiments provide data against which this prediction can be tested. A moderately beaten high α-cellulose content eucalypt pulp was acetylated[5] to various extents at two temperatures, 35°C. and 45°C.; the percentage of the total OH groups so acetylated will be referred to as the degree of substitution. Paper sheets were made under standard conditions from these pulps and their stress-strain relationships for simple tension determined under standard conditions of temperature and relative humidity.

If the modulus E is in fact a function of the number of hydrogen bonds being strained, then a plot of $100\ (1-E/E_0)$ against degree of substitution, where E_0 is the modulus for the virgin pulp, should give a unique relationship. The lower curve on Fig. 1 indicates that in fact this is so. However, as the theory under test predicts that E is proportional to $n^{\frac{1}{3}}$, the relationship between $100(1-E^3/E_0^3)$ and degree of substitution is of primary interest. Half the points on the upper curve represent a plot of these quantities. This relationship is consistent with the main tenet of the hydrogen bond theory, but verification is provided by the following argument.

The area under the stress-strain curve is the rupture energy (R.E.). If the theory is valid, then the rupture energy should, to a first approximation, be proportional to $(n/N)U_0$, that is, to n, where N=Avogadro's number. Thus $100\,[1-\text{R.E.}/(\text{R.E.})_0]$ should, when plotted against degree of substitution, give the same curve as that given by $100(1-E^3/E_0^3)$. The other half of the points on the upper curve of the figure were calculated from the rupture energy,

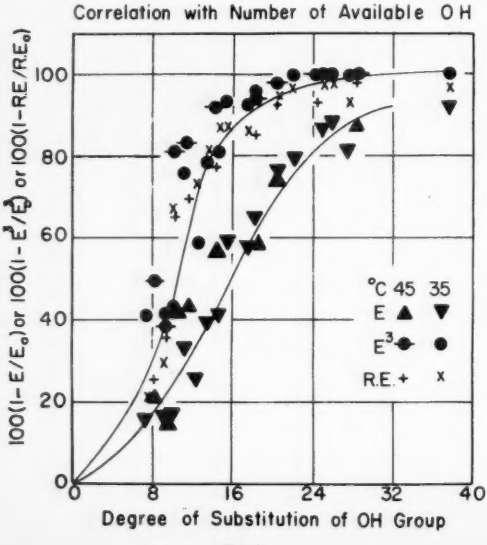

Fig. 1

and they appear to fall on the same curve as those calculated from E^3.

This curve deviates from the theory in two significant ways: (1) A linear relationship is predicted. It appears that the substitution of a hydrogen atom by a larger group not only reduces the number of hydrogen bonds but opens up the structure somewhat and, providing the process of substitution is not advanced, allows more bonds to form than would have been formed without the substitution. (2) It appears that the number of OH groups available for chemical substitution is larger than the number available for participation in those hydrogen bonds, within and between cellulose fibres, which can be deformed in the paper sheet. This is understandable as the OH groups at the surfaces of crystallites will be available for chemical reactions but will not contribute significantly to the mechanical properties, which are controlled by the amorphous fraction of the cellulose. Again, stress concentrations in the region of bonding between fibres may render the behaviour of hydrogen bonds so located of more significance during deformation than that of the bonds within the fibre. These and other details will be discussed elsewhere; the purpose of this letter is to demonstrate the validity of the basic hypothesis discussed.

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[1] Nissan, A. H., *Nature*, **175**, 424 (1955).
[2] Nissan, A. H., *Trans. Farad. Soc.*, **53**, 700, 709 (1957).
[3] Nissan, A. H., *Nature*, **178**, 1411 (1956).
[4] Nissan, A. H., *Tappi*, **41**, 131 (1958).
[5] McKenzie, A. W., and Higgins, H. G., *Holzforschung*, **10**, 150 (1956).

Fibre Surface Replication by Rolling

The communication by J. Mølgaard[1] under the above heading invites the comment that this technique was developed at least seventeen years ago in the Wool Industries Research Laboratories in Leeds. Numerous applications in the study of the surface structure of wool fibres have been published by Dr. A. B. Wildman[2] and the technique has recently attained text-book status[3]. I think it only fair to Dr. Wildman that his priority in this field should be acknowledged.

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[1] Mølgaard, J., *Nature*, **184**, 264 (1959).
[2] Wildman, A. B., 'The Microscopy of Animal Textile Fibres' (Leeds, Wool Industries Research Association, 1954); Wildman, A. B., Proc. Int. Wool Textile Research Conf., Australia, 1955, Vol. F, p. 156.
[3] Stoves, J. L., 'Fibre Microscopy', 187 (London: National Trade Press, 1957).

The precedence of Dr. A. B. Wildman's technique, pointed out by Mr. H. J. Woods is freely acknowledged; I was unfortunately unaware of his work. The principles and effect of our procedure are the same as Dr. Wildman's, though details differ.

We are applying the technique to the study of synthetic fibre surfaces which show a finer structure than the animal fibre scale structure studied by

Dr. Wildman. For this purpose, the combination of rolled fibre replicas with examination by phase-contrast microscopy, or other interference techniques, is essential[1]. This use of rolling fibre replication techniques does not appear to have been published previously.

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[1] Mart, T., and Diehl, F., *Naturwiss.*, **35**, 91 (1948). Bennett, A. H., Jupnik, H., Osterberg, H., and Richards, O. W., 'Phase Microscopy', 175, 216, 235 (New York: John Wiley & Sons Inc., London: Chapman & Hall Ltd., 1951).

METALLURGY

Chromium Distribution around Grain Boundary Carbides found in Austenitic Stainless Steel

THE usual theory proposed for the explanation of inter-crystalline corrosion of austenitic steels involves the precipitation of chromium carbides at the grain boundaries. This results in chromium impoverishment around the carbides and subsequent enhanced corrosion of the depleted region. Alternative explanations do not involve chromium depletion but postulate the precipitation of non-equilibrium carbides having low corrosion resistance[1].

With the development of the electron micro-probe analyser by Castaing[2] it has become feasible to investigate the chromium distribution around carbides formed in a sensitized austenitic stainless steel. Therefore, using the instrument available in this laboratory, a series of experiments have been carried out to test the validity of the chromium depletion theory in relation to inter-granular corrosion.

In the experiments described in this communication, Type 310 (25 per cent chromium, 20 per cent nickel) stainless steel containing 0·15 per cent carbon was given the following heat treatments. A small cube was solution-annealed at 2,100° F. for 1 hr. and water-quenched. The cube was cut in half and the two pieces held at 1,200° F., one piece for 5 hr., the other for 20 hr., each specimen being allowed to cool in air. This heat treatment produced a precipitate of chromium carbide at the grain boundaries and caused the steel to be in a sensitized condition. The chromium distribution around these carbides was then determined by scanning with the electron probe and

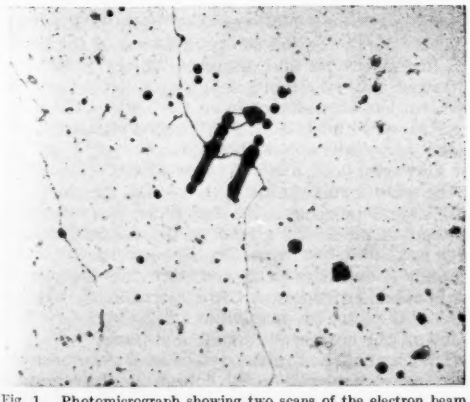

Fig. 1. Photomicrograph showing two scans of the electron beam over grain boundary carbides. Etched very lightly with glyceregia. (× 500)

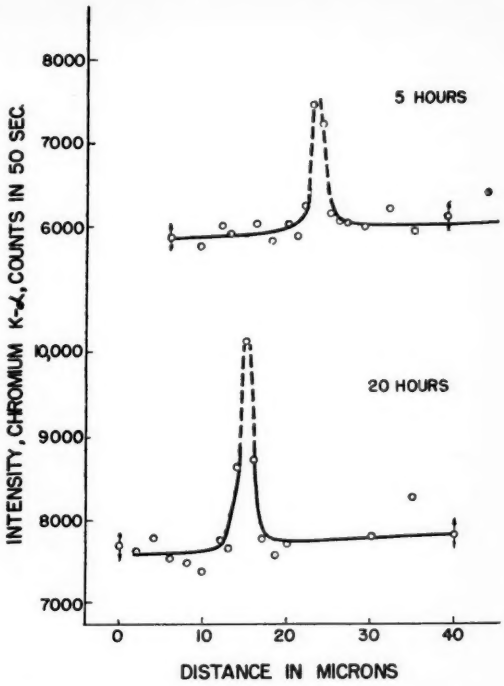

Fig. 2. Measured distribution of chromium in linear traverses of grain boundary carbides

measuring the characteristic X-ray intensities for chromium.

Fig. 1 shows the grain boundary carbides formed by the heat treatment. The traverse of the electron beam across them is indicated by the black carbon deposits resulting from the electron decomposition of vacuum pump oil. Fig. 2 is a plot of the chromium distribution found adjacent to the carbides. The measurements of chromium distribution were made with an electron accelerating voltage of 24 kV. and a beam current of 2×10^{-8} amp. Statistical fluctuation of the intensity is indicated in Fig. 2 for a range of plus or minus two standard deviations of the count; or about 2 per cent of the amount of chromium present in the irradiated spot. Under these conditions one could expect to find evidence for chromium depletion of as little as 0·5 per cent if it extended over a linear dimension of one micron. It is clearly seen from Fig. 2 that no chromium depletion can be observed in either the 5- or 20-hr. specimens. Therefore since the electron beam has an effective diameter of about 1μ, the dimensions of a chromium-depleted zone (if present) must be much less than 1μ.

The alloys used in the above experiment were given the standard corrosion test in boiling 65 per cent nitric acid to determine if sensitization had occurred during the heat treatment at 1,200° F. Corrosion-rates of 5·2 mils/month for the 5-hr. and 7·8 mils/month for the 20-hr. specimens were obtained. In comparison with the corrosion-rate of less than 2 mils/month for steel in the unsensitized condition these results indicate that both specimens are sensitized to inter-granular attack. This conclusion was also confirmed by visual observation of the specimens after testing.

Therefore, it appears justified to conclude from these results that chromium depletion around carbides in sensitized Type 310 austenitic stainless steel is very

shallow or confined to a distance of less than 1μ from the carbide precipitates.

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[1] Shvartz, G. L., and Kristal, M. M., "Corrosion of Chemical Apparatus", p. 84. Translated from Russian by Consultants Bureau Inc., New York (Chapman and Hall Ltd., London, 1959).
[2] Castaing, R., Ph.D. Thesis, University of Paris (1951). Castaing, R., Philibert, J., and Crussard, C., *Trans. Amer. Inst. Met. Eng.*, **209**, 389 (1957).

CHEMISTRY

Unexpected Formation of Volatile Compounds on Gas-Liquid Chromatography Columns

When odorous compounds of food or other products, after initial concentration and separation from the non-volatile material, are investigated by gas-liquid chromatography, only in exceptional cases can the complex mixture be completely separated on a single stationary phase.

In such instances the groups of compounds not fully separated or the compounds suspected of concealing one or more others with the same retention time, are trapped by cooling, and re-run on another stationary phase. A better separation in the second run or a splitting of a single peak from phase 1 into two or more peaks on phase 2 is thus often obtained[1].

In our experiments using this technique, unaccountable irregularities were occasionally observed with regard to the retention times before and after trapping, and aspects of this phenomenon were further investigated in some detail.

The columns used in the following experiments consisted of glass tubes, 9 ft. in length, 5 mm. inner diameter, filled with 25 per cent polyethylene glycol 600 on an acid-washed (hydrochloric acid) ground firebrick (30–60 mesh) support. Filling material in our laboratory is as a rule prepared in bulk in advance, kept in closed bottles in the dark at room temperature and used for the preparation of new columns as required; samples of the filling material used in the experiments below were 3–4 months old. Nitrogen, after passing through a column of synthetic zeolite (molecular sieve *A* 3) and magnesium perchlorate, was used as carrier gas at a rate of 35 ml./min. The temperatures of the column and of the brass two-filament hot-wire katharometer were 70° C. and 63° C. respectively in all experiments.

When samples of pure methanol, ethanol, or propanol, large with respect to the detecting characteristics of the instrument, were run on very recently renewed columns, of which, therefore, the separating efficiency was still high, we obtained low, wide, flat-topped peaks just ahead of the peak for the alcohol proper, but not fully separated from it.

After trapping the whole of the first wide peak in a trap cooled with a mixture of solid carbon dioxide and acetone and re-running the condensate on the same column, a very clear and sharp peak was obtained with a retention time corresponding to the retention time of the front edge of the original wide peak (Fig. 1).

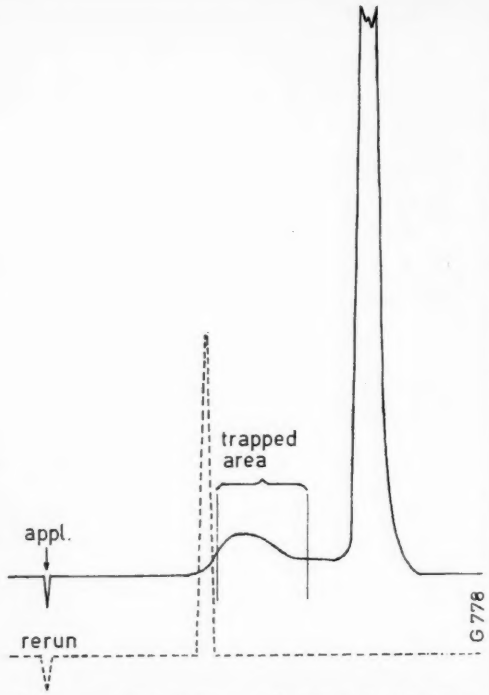

Fig. 1. ——, ethyl alcohol on polyethylene glycol gas-liquid chromatography column; — — —, re-run of trapped compound on same column

The compound causing this peak in the second run was trapped again—this time in a gas cell—and investigated by infra-red spectrophotometry. Mr. R. Belz and Mr. J. Heins, of this Institute, are thanked for their help with the infra-red identification.

The trapped compounds could thus be identified as methyl, ethyl, and propyl formate respectively, depending on the alcohol brought on the column. The alcohols used in these experiments could not be shown to contain any formate impurities. When, in test experiments, 1 per cent formate is added to the alcohol, a fine sharp peak appears on the front edge of the wide flat peak mentioned above.

In other experiments where columns were used of which the separating efficiency had decreased through use, virtually the same observations were made. The recording of the peaks was even more misleading in this case, since the flattish peak ahead of the peak of the alcohol proper had joined up with the latter and appeared only as a more or less pronounced shoulder.

When the shoulder or even the main alcohol peak was trapped and re-run, two clearly separated sharp peaks were observed, with the correct retention times for the ester and alcohol respectively. The alcohol in the second run again shows a small shoulder.

It should be mentioned that when a much smaller amount of alcohol is added to the column than had been applied in the experiments described, the formate scarcely shows as a peak and may only be seen as a slight raising of the base line starting at the retention time of the ester in question and finishing at, or just in front of, the corresponding alcohol peak.

The form of the formate peaks as they appear when the alcohols are used on the column, and their anomalous retention time, suggest the formation of the corresponding esters from breakdown products of the

stationary phase and of the alcohols during the passage of the alcohols through the column. To substantiate this tentative explanation the nitrogen leaving a column which had not been charged with sample was passed through an aqueous solution of morpholine overnight. The solution was concentrated under reduced pressure and analysed by paper chromatography[2]. One single spot of formic acid was found on the finished chromatogram.

Whether the unexpected, and somewhat disquieting results of these experiments were caused by our experimental technique which, however, does not show any uncommon aspects, or by any of the particular samples of support or stationary phase (both obtained from well-known manufacturers in the field) has still to be established. Using another brand of fire-brick support or employing hydrogen as the carrier gas gave the same results. If the phenomenon should thus prove to occur more generally, a warning seems appropriate since the occurrence of ester formations on some types of gas-liquid chromatography columns may occasionally lead to the identification of compounds in complex mixtures of isolated volatiles which are, in fact, not present in the original sample.

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[1] Corse, J., and Dimick, K. P., "Flavor Research and Food Acceptance", p. 302 (New York and London, 1958).
[2] Osteux, R., Guillaume, J., and Laturaze, J., *J. Chromat.*, **1**, 70 (1958).

Transition of Calcium Sulphate Hemihydrate to Insoluble Anhydrite

Recently Powell[1] directed attention to the fact that for calcium sulphate hemihydrate the rate of transition of soluble anhydrite to insoluble anhydrite was greater in the α- than in the β-form. Similar phenomena were observed in our experiments[2].

Hemihydrate was heated at temperatures ranging from 120° C. to 170° C. for various periods of time in a thermo-balance, and then kept in air for more than a week. The quantity of insoluble anhydrite formed from the soluble form was estimated from the weight increment due to the natural rehydration of the untransformed soluble material on leaving it exposed to the air. Fig. 1 shows the results obtained for heating at 170° C. The ordinate represents the weight percentage of the insoluble anhydrite and the abscissa the time of heating at 170° C. after dehydration was complete. The following kinds of hemihydrate were examined.

A. Hemihydrate prepared by heating a kind of gypsum, a by-product in the manufacture of salt, in an autoclave; granular crystals about 30 μ across.

B. Ordinary plaster of Paris prepared by heating the same material as in *A* in air; fine crystals with shapes difficult to determine under the microscope.

C. A kind of plaster of Paris manufactured commercially; the crystal state is similar to *B*.

D and *E*. Hemihydrate prepared by heating the dihydrate obtained by setting *B* or *C* in an autoclave; fine and rather needle-like crystals about 1 μ in width.

Afterwards, another series of measurements was made on hemihydrate *F*. The procedure was almost the same as above except that a smaller sample was used and the thermo-balance had a flat tray in order to make samples more sensitive to heat. The results obtained are shown in Fig. 1 by broken lines. Curves *F–R* and *F–S* correspond to rapid heating, taking about 5 minutes from 80 to 170° C. and to slow heating taking about 65 minutes, while about 60 minutes were required in the previous measurements. The curve *F–G* indicates the case of heating fine powder of hemihydrate *F*, ground in a mortar, under the same condition as *F–R*.

F. Hemihydrate prepared by heating natural lump gypsum which consists of fine crystals in an autoclave; granular crystals somewhat larger than *A*.

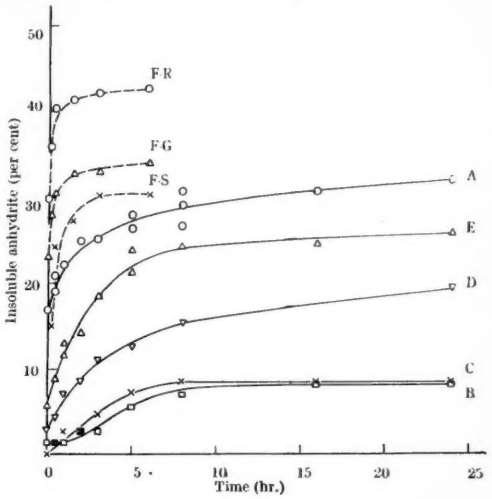

Fig. 1 shows that the hemihydrates prepared in an autoclave form insoluble anhydrite faster than ordinary plaster of Paris. This result agrees with that of Powell. However, there are considerable differences in the rate of formation among the various kinds of hemihydrate prepared in an autoclave. In addition, the rate decreases when fine powder is used or when the rate of heating up to 170° C. is lowered. From these results, it seems that the rate should be considered in connexion not only with the types of hemihydrate, which are assorted mainly with the methods of preparation, but also with the crystal size or faults as described by Buerger[3].

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[1] Powell, D. A., *Nature*, **182**, 792 (1958).
[2] Mitsuki, C., and Kita, M., *Reports Gov. Ind. Res. Inst., Nagoya*, **9**, 413 (1955).
[3] Buerger, M. J., "Phase Transformation in Solids", 206 (Wiley, New York, 1951).

Reduction of the $FeS_2O_3^+$ Ion at the Dropping Mercury Electrode

The technique of capacity flow[1], admirably adapted to the study of transient compounds, demands complete homogeneity of the contents of the reaction cell, in contradistinction to the technique of polarography, which depends on there being a concentration polarization. Exploratory experiments showed, however, that the two techniques could be combined, and adequate polarograms obtained, in solutions stirred by rapid

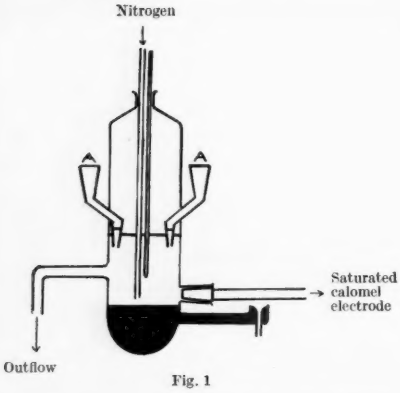

Fig. 1

streams of nitrogen; under these conditions the capacity flow technique could be used to hold the concentration of transient intermediates, such as the thiosulphatoferric ion ($FeS_2O_3^+$) constant, so that its reduction might be studied.

The vessel used to study this ion is shown in Fig. 1. The solutions under investigation flowed in through the entrance tubes *A*–*A*, while a fairly brisk stream of nitrogen was bubbled through the liquid in the vessel. The mercury and reactant solution-levels were held constant by the outflows provided. A mercury electrode drop-rate of 1 per sec. was used, and the potentials were measured against a saturated calomel electrode.

The current-voltage curve I was obtained with a 10 m*M* solution of mixed Cd^{++}, Zn^{++}, Mn^{++}, Fe^{++} and Fe^{+++} ions in 1 *M* potassium chloride. This solution was not rigorously freed from dissolved air. The experiments on the complex ion were carried out by running in solutions of *M*/40 ferric nitrate and sodium thiosulphate, both in 1 *M* potassium chloride, at roughly equal rates. The resulting concentration of $FeS_2O_3^+$ would be expected to be about $9 \times 10^{-3}\,M$[2]. The first runs showed only Fe^{++} and Fe^{+++} waves, and a hump at −0·1 V. This hump was not due to dissolved air, for it was not observed with either solution when examined alone, nor did prolonged bubbling of nitrogen affect it. A resolution of the hump could be achieved by the addition of surface active agents such as 'Lissapol *N*', or methyl red, when the current-voltage curve II was obtained.

The wave at −0·18 V. is not to be confused with the thiosulphate wave at about −0·15 V. observed by Kolthoff and Miller[3] using potassium nitrate as supporting electrolyte, for experiments in the present apparatus with the thiosulphate alone showed no such wave, and the occurrence of the observed wave only in the mixed solutions suggests strongly that it is due to the transient ion $FeS_2O_3^+$.

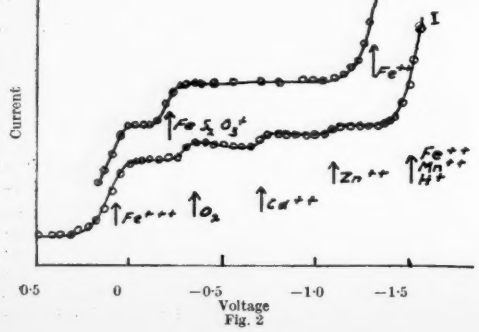

Fig. 2

This work was carried out at the Akers Research Laboratories of Imperial Chemical Industries.

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[1] Denbigh and Page, *Disc. Farad. Soc.*, **17**, 145 (1954).
[2] Page, *Trans. Farad. Soc.*, **50**, 120 (1954).
[3] Kolthoff and Miller, *J. Amer. Chem. Soc.*, **63**, 1401 (1941).

Influence of Counterion Association on the Configurational Stability of Deoxyribonucleic Acid in Solution

There is strong evidence that the double stranded helix proposed by Crick and Watson on the basis of the X-ray diffraction photograph of deoxyribonucleic acid fibres[1] is present also in solution[2].

It must be assumed, therefore, that even in solution the electrostatic repulsion between the negatively charged phosphate groups in the two strands is largely balanced by the hydrogen bonds and other, less specific, attractive interactions between the bases.

However, owing to the essentially anionic polyelectrolyte character of deoxyribonucleic acid, the negative charges on the macroion may be partially screened by the sodium counterions or other added ions, and therefore the electrostatic repulsions may be varied by changing the ionic strength. An increase in electrostatic repulsion between the two strands brought about by lowering the ionic strength may therefore be expected to cause a disordering of the structure similar to that produced by heat treatment or *p*H changes[3,4]. We have investigated the possible occurrence of 'dilution-denaturation' by studying the ionic activity and mobility of the counterions, and the ultra-violet absorption, as a function of dilution of sodium deoxyribonucleic acid concentration in the absence of added salt.

Several samples of sodium deoxyribonucleic acid obtained from calf thymus were examined. A few had been prepared in our laboratory according to the method of Kay *et al.*[5], others were supplied by the Nutritional Biochemical Corporation, Cleveland, and a very pure sample was supplied by Dr. V. Luzzati, Centre de Recherches sur les Macromolécules, Strasbourg.

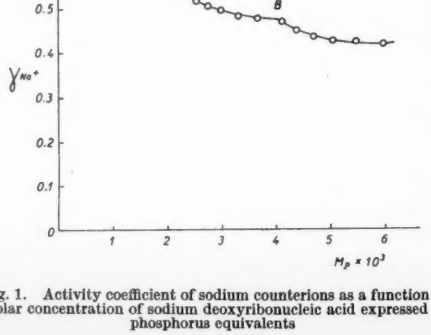

Fig. 1. Activity coefficient of sodium counterions as a function of molar concentration of sodium deoxyribonucleic acid expressed as phosphorus equivalents

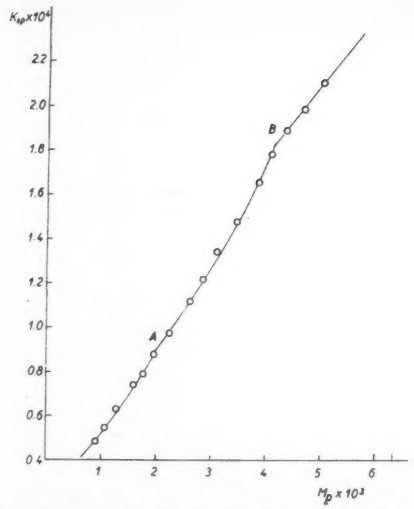

Fig. 2. Specific conductivity as a function of sodium deoxyribonucleic acid concentration expressed as phosphorus equivalents

In Fig. 1 the activity coefficient of sodium ions determined with a membrane electrode[6,7] is plotted as a function of concentration of deoxyribonucleic acid. Fig. 2 shows a plot of the specific conductivity.

Two critical concentrations may be clearly recognized in the curve of Fig. 1 and less evidently in the curve of Fig. 2 (point A and point B). Point A disappears in presence of $2 \times 10^{-3} M$ of sodium chloride while point B is shifted to a lower concentration. Actually, the two discontinuities occur in the same range of concentrations in the conductivity and the activity coefficient curves, but not at the same positions. Although less sharp, these discontinuities are of the type observed in solutions of colloidal electrolytes at the critical micelle concentration[8,9]. The lower critical concentration (point A) is in the same concentration range where deviation from the Lambert-Beer law at 260 mμ begins. This effect has already been observed by Cavalieri *et al.* who have taken it as an indication of dilution-denaturation[4].

As far as point B is concerned, it occurs in a range of concentration where X-ray studies indicate the presence of small aggregates of rod-like particles[10]. Considering the double stranded helices as molecular units, this second critical point should correspond to an intermolecular aggregation. This appears consistent with the shifting of point B toward lower concentrations in presence of added salt, as is usually observed for the critical micelle concentration of colloidal electrolytes.

In light of the present results, the events taking place on diluting a concentrated solution of deoxyribonucleic acid may tentatively be schematized as follows:

In a very concentrated solution, small aggregates of deoxyribonucleic acid double stranded helices are present. The structure of these aggregates may be described according to Riley and Oster[10] as hexagonally close packed micelles of rod-like particles with a large fraction of counterions in the interior or near the surface of the micelles. This distribution tends to lower both the mobility and the activity of the counterions considerably.

Complete dissociation into isolated molecules occurs around point B, which is similar to a critical micelle concentration. Upon further dilution, the fraction of counterions held closely or bound to the macroions tends to decrease, and a progressive charging up of the two strands takes place. This results in an increase of the electrostatic repulsion between the negatively charged phosphate groups in the two strands, leading to occasional fissions of hydrogen bonds along the double helix. The consequent partial separation of the two strands and the formation of disordered sections with a lower charge density in turn decreases the binding of counterions, thus accelerating further hydrogen bond ruptures. When the residual fraction of unbroken hydrogen bridges is no longer sufficient to hold the two strands together in an orderly fashion, the structure finally collapses. This event would occur roughly around point A, which should be considered as a kind of critical concentration for dilution-denaturation. This process is prevented by added small ions which act as a protective screen against dilution-denaturation.

Experiments have shown that the critical concentrations may vary with the state of the sample of deoxyribonucleic acid and its mode of preparation. In fact, the so-called 'native state' of deoxyribonucleic acid might be regarded almost as an 'ideal state'. Full details of this work will be published elsewhere.

This work was sponsored and financially supported by Colgate-Palmolive Co., New York, which is gratefully acknowledged. We are also indebted to Prof. G. Giacomello for his continuous interest and Prof. P. Doty and Dr. M. F. Perutz for critical reading of the manuscript.

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[1] Crick, F. H. C., and Watson, J. D., *Nature*, **171**, 737 (1953).
[2] Doty, P., Special Issue of Collection of Czechoslovak Chemical Communications, **22**, 5 (1957).
[3] Laland, S. G., Lee, W. A., Overend, W. G., and Peacocke, A. R., *Biochim. Biophys. Acta*, **14**, 356 (1954).
[4] Cavalieri, L. F., Rosoff, M., Rosenberg, B. H., *J. Amer. Chem. Soc.*, **78**, 5239 (1956).
[5] Kay, E. R. M., Simons, N. S., and Dounce, A. L., *J. Amer. Chem. Soc.*, **74**, 1724 (1952).
[6] Botré, C., Crescenzi, V., Mele, A., Proc. Intn. Symposium Coordination Compounds, Rome 1957, Suppl. *Ricerca Scientifica*, **28**, 369 (1958).
[7] Ascoli, F., Botré, C., Crescenzi, V., Liquori, A. M., and Mele, A., *J. Polymer Sci.* (in the press).
[8] Botré, C., Crescenzi, V., Mele, A., *J. Phys. Chem.*, **63**, 650 (1959).
[9] Harkins, D., "The Physical Chemistry of Surface Films", (Reinhold Publ. Co., New York, 1957).
[10] Riley, D. P., and Oster, G., *Biochem. Biophys. Acta*, **7**, 526 (1951).

BIOCHEMISTRY

Evidence of Side-Chain Cleavage during the Reaction of Epinephrine and Norepinephrine with Ethylene Diamine

In a previous communication[1], we reported that several products are obtained from the condensation of epinephrine and norepinephrine with ethylene diamine according to the Weil-Malherbe and Bone procedure[2]. Recently Yagi and Nagatsu[3] have suggested that the main product from norepinephrine in this reaction is identical with a secondary product of epinephrine probably by a process of demethylation of the latter.

Table 1. Physical characteristics of products A, B, C, and D

Spot	Fluorescence	R_F values (2nd dimension in parentheses) from epinephrine	from norepinephrine	from catechol	from 3, 4-dihydroxy-benzaldehyde	Radioactivity (from α-^{14}C-DL-epinephrine)
A	yellow green	0·41 (0·63)	0·43 (0·66)	0·42 (0·64)	0·42 (0·63)	none
B	yellow brown	0·41 (0·55)	0·42 (0·55)	0·42 (0·54)	0·42 (0·55)	none
C	buff	0·51 (0·37)	0·52 (0·39)	0·52 (0·38)	0·51 (0·39)	none
D	blue green	0·60 (0·27)	0·58 (0·28)	missing	missing	positive

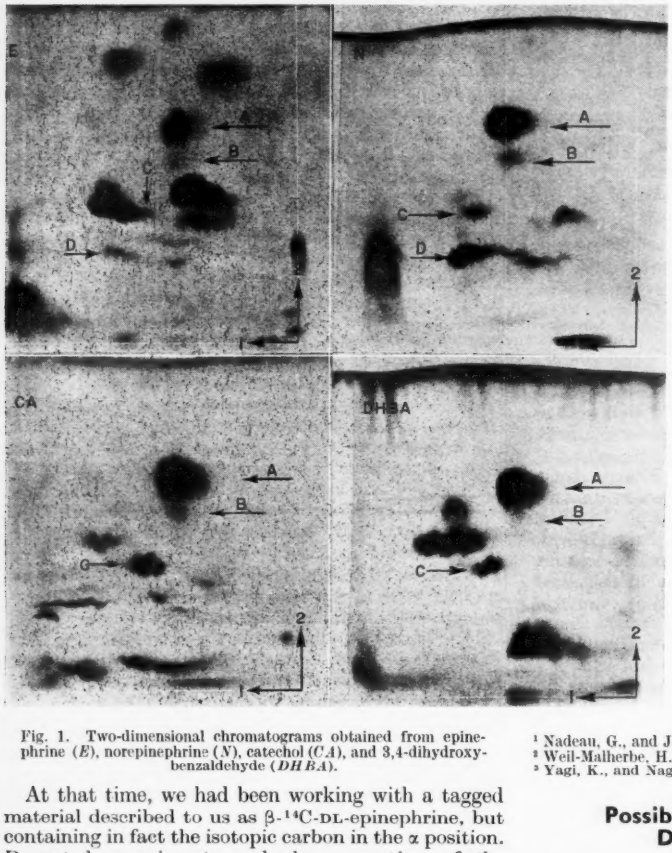

Fig. 1. Two-dimensional chromatograms obtained from epinephrine (*E*), norepinephrine (*N*), catechol (*CA*), and 3,4-dihydroxybenzaldehyde (*DHBA*).

At that time, we had been working with a tagged material described to us as β-^{14}C-DL-epinephrine, but containing in fact the isotopic carbon in the α position. Repeated experiments and closer scrutiny of the chromatograms obtained by the procedure already described[1] revealed that at least three of the fluorescent spots (*A*, *B*, and *C* in Fig. 1*E*) were not radioactive, and, hence, that part of the epinephrine had actually undergone a complete rupture of the side chain at one time of the reaction. In order to verify this hypothesis, chromatograms obtained from catechol and ethylene diamine were compared with those of epinephrine and norepinephrine. The three fluorescent spots *A*, *B*, and *C*, were found to be common to the three chromatograms, while a fourth one, *D*, was limited to epinephrine and norepinephrine (Fig. 1 and Table 1). Since the latter was radioactive (from tagged epinephrine), it suggested a cleavage somewhere along the chain further than α-carbon, either between α- and β-carbons or by N-demethylation.

The experiment was repeated with 3,4-dihydroxybenzaldehyde and with 3,4-dihydroxybenzoic acid, only to reveal again the same three spots *A*, *B* and *C*. Since these spots had been obtained with catechol, it implied decarboxylation of the acid (or of the aldehyde after a Cannizzaro-like reaction). This behaviour did not, of course, exclude the possibility of an α-β-cleavage as an intermediate step in the case of the catecholamines.

These various experiments do not agree with the statement by Yagi and Nagatsu who have attributed the similarity of the main product of norepinephrine and of a by-product of epinephrine (spot *A* according to their mode of separation) to N-demethylation of the latter, but are much more compatible with a rupture of the side chain itself. Their hypothesis, however, remains valid if applied to fluorescent spot *D* which is common to both catecholamines, is radioactive from tagged epinephrine, but missing from catechol, from 3,4-dihydroxybenzaldehyde (Fig. 1 and Table 1) or from 3,4-dihydroxybenzoic acid. Further experimental details will be reported at length elsewhere.

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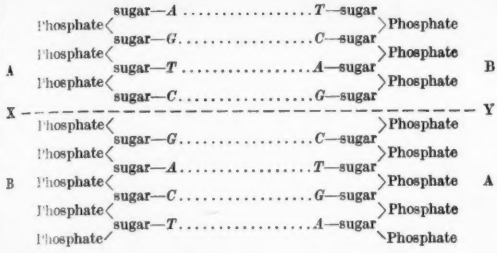
[1] Nadeau, G., and Joly, L. P., *Nature*, **182**, 180 (1958).
[2] Weil-Malherbe, H., and Bone, A. D., *Biochem. J.*, **51**, 311 (1952).
[3] Yagi, K., and Nagatsu, T., *Nature*, **183**, 822 (1959).

Possible Simplified Structure of Deoxyribonucleic Acid

Although the Watson-Crick model[1,2] provides a plausible explanation of the structure and replication of deoxyribonucleic acid, there remains one feature of it which, in our opionion, is unnecessarily complex. In the classical model two complementary chains of nucleic acid are intertwined. Each has a fixed order of base sequence such that base pairing between adenine-thymine and cytosine-guanine occurs along the double helix. During replication the two chains uncoil and each acts as a template for the synthesis of the other.

The postulated existence of this duplex type of nucleic acid in Nature seems to us unnecessary, and we accordingly propose a scheme whereby the requirements of the Watson-Crick model can be met using only a single type of nucleic acid chain.

In this scheme both polynucleotide chains of the double coil are identical. Each half of any single polynucleotide chain is moreover complementary to the other half. Diagrammatically the model may be represented as follows where *A* = adenine, *T* = thymine, *G* = guanine and *C* = cytosine.



The broken line X–Y represents a dividing line in each polynucleotide chain, such that portion B always has a base sequence complementary to portion A and could fold back on itself to give the correct base pairing. Each chain as a whole is identical to the other and in consequence during cell division each individual deoxyribonucleic acid chain will exactly replicate itself. For the particular segment shown in the diagram the sequence will be invariably *A–G–T–C–G–A–C–T*. In general, the one-half of a parent deoxyribonucleic acid chain may have any arbitrary base sequence, but the sequence of the second half must be complementary to the first.

Unlike the Watson-Crick model this scheme does not require the existence of two different, though complementary, chains for each type of acid in a cell. It does however require that each deoxyribonucleic acid chain be composed of an even number of bases. The scheme would account for a double helical structure of the acid during cell division, but also permits of the existence of single deoxyribonucleic acid chains during resting periods. A similar duplex model consisting of identical deoxyribonucleic acid chains but with pairing between identical bases has been suggested by Donohue and Stent[3]. Their model does not however account for the X-ray diffraction results obtained with deoxyribonucleic acid from a variety of sources.

The scheme proposed would account for the existence of a plane of symmetry in living organisms, and might also explain the symmetrical division of chromosome material during spermatogenesis.

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[1] Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 737, 964 (1953).
[2] Perutz, M. F., *Endeavour*, **17**, 190 (1958).
[3] Donohue, J., and Stent G. S., *Proc. U.S. Nat. Acad. Sci.*, **42**, 734 (1956).

Measurement of Inhibition by Azide in Biochemical Assay Systems involving Nitrite Estimation by Diazotization

Villanueva[1] recently directed attention to the interference by azide in diazotization procedures used in assay systems. He suggested that the reported inhibition by azide of numerous enzyme systems in which nitrite production has been measured might require re-investigation.

We have independently reached this conclusion during a study of nitrate reduction by the aldehyde oxidase system of potato, and our method for dealing with this point may be of interest in connexion with the re-measurement of inhibition by azide in nitrate reductase and xanthine or aldehyde oxidase systems.

First attempts to test for azide inhibition of a nitrate reduction system showed that nitrite reacts immediately with azide when the acidified sulphanilamide reagent is added in the diazotization step. This reaction is probably that shown by Partington[2]:

$$NO_2^- + N_3^- + 2H^+ \rightarrow N_2 + N_2O + H_2O$$

Azide at concentrations normally used in inhibition tests (10^{-4}–10^{-3} *M*) caused proportional losses of nitrite estimated in standard solutions, and almost total losses of nitrite produced enzymically in concentrations of the order of $2–5 \times 10^{-5}$ *M*. The results given by Villanueva show that in his system containing azide at a final concentration of $3 \cdot 3 \times 10^{-3}$ *M*, where 92 per cent interference was observed, the concentration of nitrite was approximately $1 \cdot 2 \times 10^{-3}$ *M*.

The reaction between azide and nitrite can, however, be applied in the manometric estimation of the latter when produced in enzymic reactions. The effect of inhibition of azide is then determined by comparison of evolution of gas produced in the reaction between nitrite and azide in acid solution when azide is added at the end of the assay period or initially also, when present as an inhibitor. In practice, 0·5 ml. of 10^{-2} *M* azide solution in one side-arm was added at the end of the assay period to the reaction mixture containing 3·5 ml. of 0·13 *M* phosphate buffer *p*H 6·0 in the main compartment and 0·4 ml. of 2*N* hydrochloric acid was added from a second side-arm to produce rapid gas evolution. The enzymic reaction was started by adding 0·1 ml. of *M* acetaldehyde solution by dislodging a glass cup hooked on the centre well.

The following points merit note. As nitrous oxide is appreciably soluble in water, the volume of gas measured is less than the theoretical volume. In our experiments, 2μ moles nitrite yielded 82–86 μlitres of gas at 27°C. in an atmosphere of nitrogen instead of 89·6 μlitres. Manometric estimation of nitrite production using azide is nevertheless more sensitive than that using sulphamic acid proposed by Robinson[3], as nearly twice as much gas is evolved. Sodium azide is unstable when acidified, and yields variable small amounts of gas, depending on initial concentration, which must be accounted for so far as possible in blank tests, since azide is in excess of the equivalent amount of nitrite. The activity of the uninhibited system should also be estimated independently by a colorimetric determination of nitrite in an aliquot from a parallel assay mixture incubated and shaken under conditions identical with those used in the manometric procedure.

Using the procedures outlined, we have shown that aldehyde oxidase of potato is inhibited 80–90 and 45 per cent by 1 to 5×10^{-3} and 5×10^{-4} *M* azide concentrations respectively when reduction of nitrate to nitrite is used to measure activity. The inhibition is probably immediate and is completely reversible, since dialysis for 3 hr. after incubation with azide permits normal rates of nitrite production in the usual assay system.

The details of this work will be published elsewhere.

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Oct. 15.

[1] Villanueva, J. R., *Nature*, **184**, 549 (1959).
[2] Partington, J. R., "A Text Book of Inorganic Chemistry", 5th edit., p. 562 (Macmillan, London, 1946).
[3] Robinson, J., *Arch. Biochem. Biophys.*, **52**, 148, (1954).

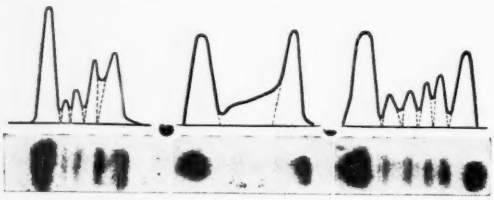
Effect of Ultrasound on the Protein of Human Serum Denatured with Tannin

Tannin—the natural tannin glycoside ester which occurs in many plants—is a weakly acidic compound. An aqueous solution of tannin gives an insoluble precipitate with solutions of salts of heavy metals, and with solutions of organic substances, such as alkaloids, gelatine and protein. Numerous investigators have studied these complexes, using various means to break them up. We have used ultrasonic vibration, with which we previously broke up the tannin-alkaloid complex in an alcoholic extract quinine bark.[1] Other workers[3] have studied the effect of ultrasound on the structure of organic substances, particularly molecular size. The present work was concerned with the complex of tannin with human serum protein.

To 0·3 ml. blood serum was added 0·3 ml. 1 per cent aqueous solution of tannin. The complex was then subjected to ultrasound for 30 minutes. A piezoelectric generator with frequency 780 kc./s. and power 30 W./cm.^{2} was used. A stream of water kept the temperature in the range 12·5–17°C.

The serum was then investigated by electrophoresis on Whatman No. 1 filter paper, with veronal buffer *p*H 8·6 and ionic strength 0·10. 8 μl. serum were placed on the strips and electrophoresis was carried out for 16 hours at 8 V./cm. (0·19 m.amp./cm.-wide strip).

Electrophoresis patterns of normal serum, the complex with tannin, and the complex after the action of ultrasound are shown in Fig. 1.



(*a*) (*b*) (*c*)

Fig. 1. Electrophoresis of human serum protein. (*a*), Normal; (*b*), after treatment with tannin; (*c*), as (*b*) after 30 min. treatment with ultrasound.

Our results suggest that tannin forms a permanent insoluble complex with the serum globulins rather than with albumen. Globulins bound with tannin do not migrate in the electric field and remain on the starting line, which indicates that they are denatured by tannin. The complex appears to be broken up by ultrasound, since after this treatment we found four globulin fractions migrating at the speeds of normal α_1-, α_2-, β-, and γ-globulins. Treatment for 30 minutes did not break up all the complex, since some protein remained on the starting line. After the break up of the complex under the influence of ultrasound the size of α_1- and α_2-globulin fractions increased slightly while there was a slight decrease in the β- and γ-globulin fractions (Fig. 1). This suggests that some change in structure takes place, but it does not appear to be fundamental. The albumen fraction also appears to be slightly affected.

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[1] Adamski, R., and Mizgalski, W., *Acta Pol. Pharm.*, XIV (2), 119 (1952).
[2] Auerswald, W., and Bornschein, H., *Naturwiss.*, **37**, 524 (1950).
[3] Kanig, K., and Künkel, H., *Hoppe-Seyler's Z. Physiol. Chem.*, **309**, 162, 166, 171 (1958). Kanig, K., *ibid.*, **309**, 176 (1958).

A New Two-Dimensional Paper Chromatographic Method applied to the Separation of Amino-acids

One of the advantages of the ion-exchange cellulose papers as chromatographic media is their ability to exhibit both ion-exchange and cellulosic properties. Thus, it has been shown that ion-exchange separations can be combined with those of a partition type as a two-dimensional chromatogram to form a rapid and efficient means of resolving mixtures of inorganic ions[1].

This method has been applied to the separation of synthetic mixtures of amino-acids. The success of the method depends largely on the ability to suppress the ion-exchange characteristics of the cellulosic materials, in such a way that a purely partition separation of amino-acids can be made to take place in one direction. This has been achieved by using developing conditions under which either the functional groups of the exchange material are no longer ionized or, under which the amino-acids carry the same charge as the exchanger.

A two-dimensional separation on cellulose phosphate paper, which is a medium strength cation exchange material, is illustrated in Fig. 1. The amino-acid solution was applied to the dry paper as a single large spot and development was carried out according to conventional techniques by a descending method. In the partition solvent, *m*-cresol/ammonia, the amino-acids were anionic and separated as on unmodified cellulose. The separated spots on this and all other chromatograms were detected by spraying with an 0·2 per cent solution of ninhydrin in chloro-

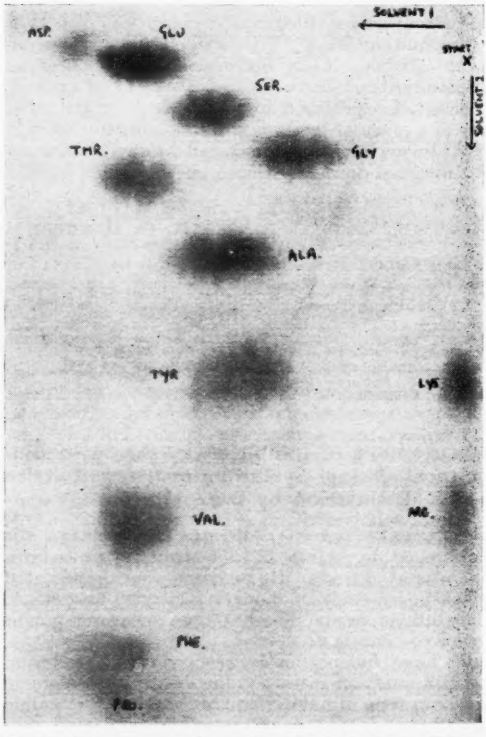

Fig. 1. A two dimensional separation of amino-acids on cellulose phosphate paper in the hydrogen form. Solvent 1:0·02*M* sodium buffer at *p*H 4·7. Solvent 2:*m*-cresol/1 per cent ammonia.

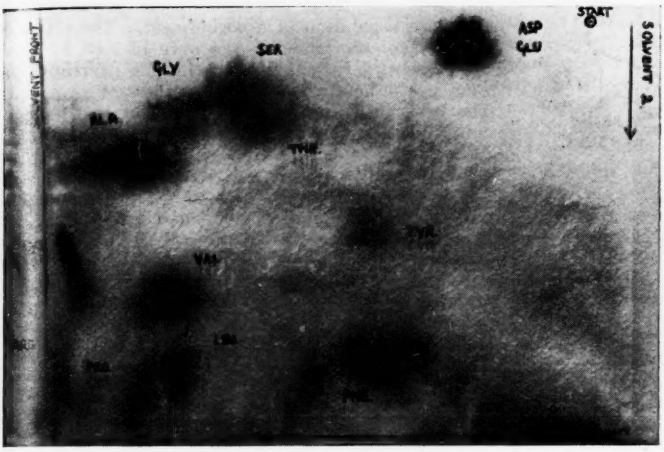

Fig. 2. A two-dimensional separation of amino-acids on *DEAE* cellulose paper in the free base form. Solvent 1:0·02*M* acetate buffer at *p*H 7·5. Solvent 2:*m*-cresol/1 per cent ammonia.

form containing 10 per cent acetic acid. Compared with conventional techniques, the overall time for this separation was cut by more than half.

In conditions where it is desirable first to wash the exchange material with the developing buffer, the amino-acid solution may be applied to the wet sheet and the first development carried out without the necessity of removing the paper from the cabinet. This further adds to the simplicity of the method.

Mixtures of organic solvents and buffer have also been used on the ion-exchange celluloses in place of the buffer alone to give increased separation and sharper spot formation, though adding to the complexity of the system. For two-dimensional separations on the weak cation exchange materials, carboxymethylcellulose and cellulose citrate papers, the use of mixed solvent-buffer systems in one direction is necessary in order to obtain adequate separation of the whole range of amino-acids.

Two weak anion exchange materials, aminoethylcellulose and diethylaminoethyl cellulose have also been investigated. Work on the former material has been hindered by the background colour produced by the ninhydrin reagent. A two-dimensional separation on diethylaminoethyl-cellulose paper is illustrated in Fig. 2. The amino-acids were applied to the dry paper in slightly alkaline solution. The weak exchange characteristics of this material are in part offset by the fact that the neutral amino-acids are stronger acids than bases and separations can be effected with aqueous buffer solutions alone. In the separation shown in Fig. 2 the partition solvent inhibited the ionization of the exchange groups.

The diethylaminoethyl cellulose paper was from an experimental batch and this probably contributed to the rather diffuse spot formation but it is apparent that the ion-exchange and partition separations combine very successfully on a two-dimensional chromatogram. The order in the first direction is sufficiently different from that in the second to produce a distribution of the amino-acid spots over the whole area of the paper. This allows the separations to be carried out rapidly on relatively small areas of paper. Distinctive colours were imparted to many of the amino-acid spots on diethylaminoethyl cellulose by the ninhydrin reagent.

Compared with conventional techniques the new method offers greater flexibility together with increased speed and degree of resolution.

Restricted space prevents the inclusion of a more detailed account but this will be published elsewhere.

I should like to thank Miss A. Gibson for assistance with the experimental work, Mr. A. E. Balston for the photography and the Directors of W. and R. Balston, Ltd., for permission to publish this work.

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[1] Knight, C. S., *Nature*, **183**, 165 (1959).

Estimation of Vitamin C by Agar Diffusion

The assay of antibiotics by diffusion through agar is well known and was first described by Heatley[1]. Dingle *et al.*[2] determined the concentration of enzymes by mixing the substrate in agar and measuring the zone of reaction after applying paper disks saturated with enzyme solutions to the agar plate. In both of these methods the diffusion through the agar was proportional to the concentration of the solution applied.

Bessey[3] and others have described the use of 2,6-dichlorophenolindophenol as a specific oxidation-reduction indicator for the titration of vitamin C in biological systems. By suspending the indicator in agar a method has been devised for measuring vitamin C by diffusion. The diffusion of the vitamin was found to be a direct function of the concentration. Potassium permanganate was also used as an indicator for the diffusion method.

Agar plates containing the indicators were made in the following manner: 2,6-*dichlorophenolindophenol*, 80 mgm. of the indicator and 2 gm. of Difco agar were added to 100 ml. of water and the solution heated for 15 min. in a boiling water bath. The solution was allowed to cool to 50°C. Fifteen ml. was poured into each 3-in. square Petri dish (approximate height of agar = $\frac{1}{8}$ in.) and the agar was allowed to harden. *Potassium permanganate*, 60 mgm. of potassium permanganate plus 2 gm. of Difco agar were heated with 100 ml. of water for 15 min. in a boiling water bath. The solution was poured into plates as above.

Aqueous solutions containing 10, 5, 2·5 and 1·25 mgm. of ascorbic acid per ml. were prepared. Paper disks (Schleicher) and Schuell (No. 740-*E*, 12·7 mm.) were partially dipped into each solution. When the disks were completely saturated with solution they were placed on the agar plates. The disks were applied 30 sec. apart so that each zone could be measured after the same diffusion time. The zone diameters were measured in millimetres after 30 min. Although an ordinary rule can be used for measuring the zones, more precise measurements were made with a Spencer (Quebec) colony counter (a magnifying device in which the colony counting plate was replaced with a linear millimetre rule for measuring zone diameters). A standard curve was drawn by plotting the zone diameter against the concentration

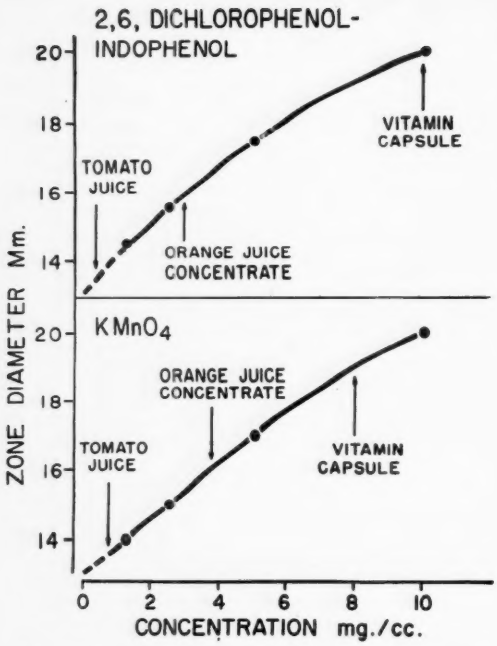

Fig. 1. Standard curves for ascorbic acid based on a 30-min. diffusion time. The zone diameters obtained with three products containing vitamin C are indicated on the curves.

of ascorbic acid (Fig. 1). A straight-line relationship was obtained in the concentration range of 1–5 mgm. per ml. with both indicators.

The time of diffusion can be varied from 15 minutes to several hours. With the permanganate indicator, diffusion times up to 20 hr. or longer may be used. However, with the indophenol indicator, diffusion times greater than a few hours leads to diffusion of the indicator back into the clear zone.

Pure ascorbic acid at *p*H 4 and at neutrality gave the same zone diameters. However, when a sample of orange juice concentrate was assayed at its natural acidity (*p*H 4) abnormally large zones were obtained with both indicators; colourless with the permanganate and pink zones with the 2,6-dichlorophenolindophenol. This increased zone size was evidently due in large part to citric acid, for it was found that pure citric acid gave zones of this type. (The 2,6-dichlorophenolindophenol dye is normally a blue colour but turns pink in acid solutions.) Canned tomato juice also gave a somewhat larger zone at its natural acidity than when neutralized. The effect of acids other than ascorbic on the indicators was eliminated when the solutions were neutralized (*p*H 6·5–7·5) before they were assayed.

A commercial vitamin capsule was dissolved in water to make a 10 mgm. per ml. solution of vitamin C. The solution had a *p*H of 6·5 and was assayed without adjustment of *p*H. The zone sizes obtained with the vitamin capsule and the neutralized juices are shown in Fig. 1. From the results obtained, it appears that the indophenol indicator is more specific for vitamin C than permanganate for in each case values closer to the known average vitamin C concentrations[4] were obtained.

Iron salts are known to interfere in the indophenol titrimetric assay method[5]. To test the effect of iron salts on the diffusion method, ferric chloride, ferrous sulphate and ferric ammonium citrate were each added to a solution of ascorbic acid. The ratios of iron salt to ascorbic acid used were 12·5:10, 5:10 and 5:10 respectively. The ferric chloride decreased the zone size indicating a 50 per cent loss of vitamin C. The other iron salts showed no effect in the assay since the zone diameters were the same as the control.

The diffusion method for assay of vitamin C has certain advantages over the titrimetric methods used heretofore: (1) since vitamin C diffuses rapidly through agar, reducing substances that diffuse less rapidly will have little effect; (2) the estimation of vitamin C can be carried out in highly coloured solutions; (3) large numbers of samples can be assayed simultaneously in a relatively short period of time.

The disadvantage of the diffusion method is its inability to measure directly low concentrations of vitamin C (that is, less than one mgm. per ml.). However, this disadvantage might be overcome by addition of a known quantity of pure ascorbic acid to the test solution so that the final concentration is in the accurate range.

The work described originated as a high school Science Fair project by the senior author and was carried out partly in the Merck Sharp and Dohme Research Laboratories.

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[1] Heatley, N. G., *Biochem. J.*, **38**, 61 (1944).
[2] Dingle, J., Reid, W. W., Solomons, G. L., *J. Sci. Food Agr.*, **4**, 149 (1953).
[3] Bessey, O. A., and King, C. G., *J. Biol. Chem.*, **103**, 687 (1933).
[4] Council on Foods of the Amer. Med. Assoc. (1939).
[5] Gawron, O., and Berg, R., *Ind. Eng. Chem.* (*Anal. Ed.*), **16**, 757 (1944).

Flavins as Components of Folic Acid Reductases

It has been demonstrated that tetrahydrofolicacid is an important intermediate in the conversion of folic acid into the functional form of this vitamin[1–3]. This conversion has been shown to take place in two steps both of which require reduced triphosphopyridine nucleotide as the hydrogen carrier[4]. The following mechanism has been formulated:

$$F + TPNH + H^+ \rightarrow FH_2 + TPN^+$$
$$FH_2 + TPNH + H^+ \rightarrow FH_4 + TPN^+$$

A 15-fold purification of the overall system reducing folic acid to tetrahydrofolic acid in chicken liver has been reported by Futterman[4].

The present study describes preparations from sheep liver which have twenty times the specific activity of the starting material. The purified enzyme system has been partially resolved into apoenzyme and the cofactor. Attempts to reactivate the resolved system have shown that flavine mononucleotide can regenerate the activity by 84 per cent. Flavine adenine dinucleotide and some of the known flavin analogues have been found to inhibit the activity of this system. The starting material employed for the purification studies was sheep liver acetone powder extracted in *p*H 5·5 phosphate buffer. The extract was fractionated with ammonium sulphate and the overall activity was found in the fraction precipitated at 50–75 per cent saturation. This fraction was dissolved in water, dialysed and then subjected to repeated adsorption on calcium phosphate gel followed by elution in phosphate buffer at *p*H 7·4. The

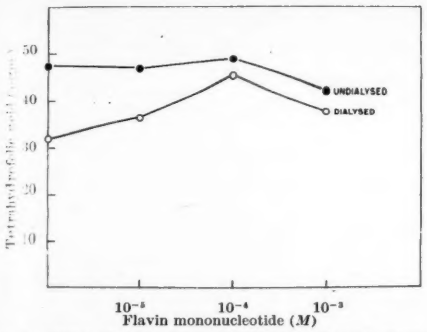

Fig. 1. Effect of flavin mononucleotide on folic acid reductases before and after resolution of the sheep liver enzyme system. The reaction mixture: 100 μgm. of folic acid; 125 μmoles phosphate buffer *p*H 5·5; 2 μmoles sodium citrate; 0·04 μmoles triphosphopyridine nucleotide; isocitric dehydrogenase extract from pig heart (10 mgm. protein); dialysed or non dialysed sheep liver preparation (2·0 mgm. protein). Total volume 2ml. Time of incubation 1 hr. Temperature of incubation 37°C.

specific activity of the product so obtained was 20 times that of the starting material. The yield was only 10 per cent. The method has been found to give consistent and reproducible results although the final yield is low. The enzyme activity was determined by assay of the diazotizable amine formed from the spontaneous decomposition of tetrahydrofolic acid[5].

In the course of studies on folic acid reductases in tumours induced in rat liver with 4-dimethylaminoazobenzene, it was found that this system behaved like many other flavin enzymes and was considerably depressed in the neoplastic tissue. Furthermore, it was found that some of the known flavoprotein inhibitors like 'Atebrine' and riboflavin depressed the activity of folic acid reductases in rat liver[6]. Following these observations attempts were made to resolve the purified enzyme system. Dialysis of the purified preparation in water saturated with carbon dioxide[7] gave consistent results.

The results in Fig. 1 show the effect of various concentrations of flavin mononucleotide on the conversion of folic acid into tetrahydrofolic acid by the sheep liver preparation, before and after dialysis in water saturated with carbon dioxide. It is evident that, in the undialysed preparation, the concentrations of flavin mononucleotide used produced very little effect. On dialysis, the activity of the enzyme system without the addition of the cofactor was decreased indicating partial resolution, and addition of $10^{-4}M$ flavin mononucleotide regenerated 84 per cent of the original activity.

Results of experiments, in which the effect of some well known inhibitors of flavo-proteins were investigated, are given in Table 1. 'Atebrine' and riboflavin, which are known to inhibit the activity of several flavoproteins[8,9], were found to inhibit the present system. In agreement with the observation that flavin adenine dinucleotide inhibits some flavin enzymes involving flavin mononucleotide as cofactor[9], it was found that pig heart concentrates of flavin adenine dinucleotide produced pronounced inactivation of folic acid reductases. In studies which are still in progress it has been possible to demonstrate, in some preparations, reversal of the inhibitory effect of riboflavin by the addition of extra flavin mononucleotide.

Table 1. EFFECT OF FLAVINS AND FLAVIN ANALOGUES ON THE FOLIC ACID REDUCTASE SYSTEM FROM SHEEP LIVER. THE REACTION MIXTURE WAS SAME AS IN FIG. 1

Type of liver preparation employed	μgm. tetrahydrofolic acid formed after addition of				
	Nil	FMN $10^{-4}M$	Riboflavin $6\times10^{-4}M$	Atebrine $10^{-2}M$	Pig heart FAD 0·5 ml.
Non dialysed	68·6	67·6	61·0	35·3	30·5
Dialysed	52·0	65·5	49·5	26·0	31·1

Experiments on the conversion of dihydro- to tetrahydro-folic acid indicate that dihydrofolic acid reductase is also activated by flavin mononucleotide.

The experimental evidence presented strongly suggests that folic acid reductases in some mammalian livers are flavoproteins in which flavin mononucleotide can function as the flavin moiety.

We wish to thank Dr. V. R. Khanolkar for the facilities made available during the course of this work.

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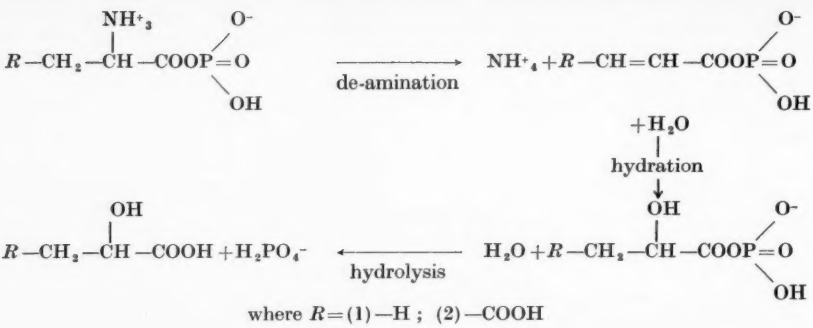
[1] Greenberg, G. R., *J. Amer. Chem. Soc.*, **76**, 1458 (1954).
[2] Silverman, M., Keresztesy, J. C., Koval, G. J., and Gardiner, R. C., *J. Biol. Chem.*, **226**, 83 (1957).
[3] Blakley, R. L., *Biochem. J.*, **58**, 448 (1954).
[4] Futterman, S., *J. Biol. Chem.*, **228**, 1031 (1957).
[5] Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, **128**, 537 (1939).
[6] Kenkare, U. W., and Braganca, B. M. (in preparation).
[7] Mueller, G. C., and Miller, J. A., *J. Biol. Chem.*, **185**, 145 (1950).
[8] Mahler, H. R., in Colowick, S. P., and Kaplan, N. O., 'Methods in Enzymology', 688 (Academic Press, New York, 1955).
[9] Wadkins, C. L., and Mills, R. C., *Fed. Proc.*, **15**, 377 (1956).

Spontaneous De-amination of Amino-acid Phosphates under Physiological Conditions

AMINO-ACIDS are stable compounds which undergo de-amination only under drastic preparative conditions. De-amination of amino-acids in aqueous solutions, at physiological *p*H, and at room temperature occurs only in the presence of enzyme systems or by irradiation. The well-known enzymatic de-aminations are oxidative de-aminations leading to the formation of keto-acids and non-oxidative de-aminations yielding fatty acids and sometimes hydroxy acids.

We have found that the phospho-anhydrides of amino-acids described previously[1] may undergo de-amination without the aid of any enzymes and are converted spontaneously into the corresponding hydroxy acids in dilute aqueous solutions. At room temperature solutions ($\sim1/100$ *M*) of glycyl, alanyl, leucyl, aspartyl and glutamyl phosphates in the *p*H range of 3–9, all give the corresponding hydroxy acids. The yield is never less than 30 per cent of the active phosphate, and depends on *p*H active phosphate concentration and the nature of the amino-acid radical. The yield of hydrolytic de-amination of the amino-acid phosphoanhydrides is strongly increased by the presence of inorganic surfaces, such as charcoal or colloidal suspensions of platinum and palladium. The yield increases with the amount of catalyst. Thus 100 mgm. of 10 per cent palladium on charcoal in 10 ml. of phosphate anhydride solution gives 100 per cent conversion to the hydroxy acid. Since the anhydrides are rapidly hydrolysed the deamination reaction must proceed at a comparable rate. The catalytic surface seems to adsorb and stabilize the phosphate end thereby to increase its chances of de-amination.

Special attention was paid to the de-amination of α-alanine phosphate to lactic acid and the de-amination of aspartyl phosphate to malic acid.



where $R = (1) —H$; $(2) —COOH$

It was shown that during de-amination double bonds appear in the reaction mixture indicating that acrylyl phosphate and fumaryl phosphate respectively are formed as intermediate steps. The above scheme is suggested for the process.

To substantiate the hypothesis, acrylyl phosphate and fumaryl phosphate were prepared from phosphorous oxychloride and the silver salts of the respective acids. They were dissolved in an aqueous buffer solution of *p*H 5. High conversions (70–90 per cent) to lactic acid and malic acid respectively were achieved in aqueous solutions (0·01 *M*) of acrylyl and fumaryl phosphate anhydride containing 10 mgm./ml. catalytic palladium (10 per cent) on charcoal.

The de-amination of glycine phosphate, with intermediary double bond formation, requires the participation of two molecules in the reaction. It has been found that two glycine phosphate molecules de-aminate to form fumaryl phosphate which afterwards hydrates to malyl phosphate. As hydrolytic end-product a high yield of malic acid is recovered.

It is worth noting that the phosphates of unsaturated acids have an enhanced tendency for reduction as revealed in the study of their oxidation-reduction potentials. This property might be related to the phenomena of reductive de-amination.

Attempts to reverse the de-amination reaction of alanine by dissolving lactyl phosphate in ammonium buffers resulted in 30 per cent yield of alanine. No unsaturation was detected however in the reaction mixture, and acrylyl phosphate did not react with ammonium ions to give alanine.

It seems, therefore, that the spontaneous de-amination of amino-acid phosphates is an irreversible process.

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[1] Katchalsky, A., and Mella Paecht, *J. Amer. Chem. Soc.*, **76**, 6042 (1954).

Hydroxylysine in Wool

Controversial results[1–5] on the hydroxylysine content of wool were due to the difficulty of analysis of hydroxylysine at very low concentrations. We have now been able to identify and estimate hydroxylysine in wool by application of new methods.

(1) *Specific Identification of Hydroxy-amino-acids on Paper chromatograms*[6]. Fig. 1 shows a descending chromatogram of a wool hydrolysate.

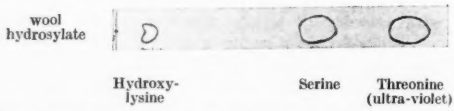

Fig. 1. Paper chromatogram of wool hydrolysate, solvent: *sec*-butanol/formic acid/water 75/15/10, developed with periodic acid

After treatment with periodic acid and subsequent spraying with acetyl acetone/ammonium acetate according to the method of Schwartz[6] two fluorescent spots of 3,5-diacetyl-1,4-dihydrolutidine appear corresponding to hydroxylysine and serine. Another spot corresponding to threonine is only visible in ultra-violet light.

(2) *High-Voltage Paper Electrophoresis.* On paper chromatograms as shown in Fig. 1 hydroxylysine runs like cystine. The cystine fractions of 50 mgm. wool were eluted, combined, concentrated, and investigated by high-voltage paper electrophoresis according to the Westphal method[7].

The electropherogram of this fraction shows the presence of hydroxylysine in wool hydrolysates (Fig. 2, *B*).

Fig. 2. Electropherogram, 4,000 V., *p*H 6·2 240 min. *A*, wool fraction; *B*, lysine and hydroxylysine; *C*, cystine; *H*, histidine; *HL*, hydroxylysine; *L*, lysine

(3) *Quantitative Estimation of Hydroxylysine with the Modified Moore and Stein Technique*[8]. Improved separation of basic amino-acids can be obtained with a column 50 cm. in height, filled with 'Amberlite' *J R* 120, particle size about 40μ. The column is eluted with 0·38 *N* sodium citrate buffer *p*H 4·26 at 30° C. and an air pressure of about 200 mm. Mercury 2-ml. fractions are collected as described by Spackman, Moore and Stein[8].

For quantitative determination of hydroxylysine 50 mgm. of wool were hydrolysed for 22 hr. with 10 ml. 6 *N* hydrochloric acid at 105°C. in sealed tubes. After evaporation of the hydrochloric acid *in vacuo* the residue was dissolved in 10 ml. sodium citrate

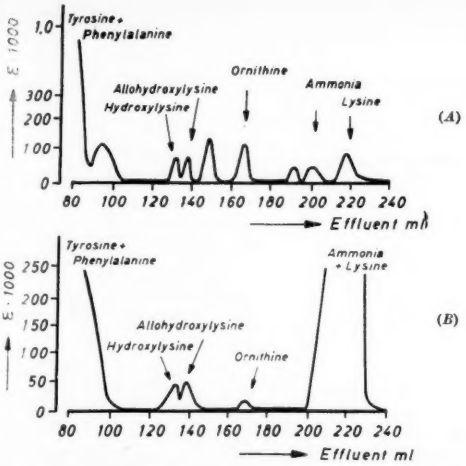

Fig. 3. *A*, Chromatogram for comparison (Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1202 (1958). Fig. 9*B*) *B*, Analysis of hydroxylysine from hydrolysate of merino wool. 30° C., *p*H 4·26, 0·38 *N* sodium citrate

buffer, *p*H 2·2. An aliquot corresponding to 19·26 mgm. of protein was used in the column. The two distinct peaks (Fig. 3, *B*) correspond to the hydroxylysine and allohydroxylysine peaks in the test chromtograms of Spackman, Moore and Stein (Fig. 3, *A*).

The values in Table 1 were obtained for hydroxylysine.

Table 1

Amino-acid	moles/gm.	Wt. (percentage)	Nitrogen (per cent of total)
Hydroxylysine ..	0·11	0·09	0·098
Allohydroxylysine ..	0·14	0·11	0·120
Total hydroxylysine	0·25	0·20	0·218

The presence of allohydroxylysine may be due to racemization at the α-carbon during hydrolysis[5]. Deviation from the given values is about ± 10 per cent, because of the low concentration of hydroxylysine in wool. The traces of ornithine found in wool probably derive from a slight decomposition of arginine[9].

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[1] Slyke, D. van, Hiller, A., and MacFadyen, D. A., *J. Biol. Chem.*, **141**, 681 (1941).
[2] MacPherson, H. T., *Biochem. J.*, **40**, 470 (1946).
[3] Middlebrook, W. R., *Nature*, **164**, 321 (1949).
[4] Simmonds, D. H., *Austr. J. Biol. Sci.*, **7**, 98 (1954).
[5] Hamilton, P. B., and Anderson, R. A., *J. Amer. Chem. Soc.*, **77**, 2892 (1955).
[6] Schwartz, D. P., *Anal. Chem.*, **30**, 1855 (1958).
[7] Werner, G., and Westphal, O., *Angew. Chem.*, **67**, 251 (1955).
[8] Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 7 (1958).
[9] Hamilton, P. B., and Anderson, R. A., *J. Biol. Chem.*, **211**, 95 (1954).

Oxidative and Phosphorylative Activities of Cytoplasmic Particles from Plant Tissue Cultures

MITOCHONDRIA have been established as the centres of respiratory and energy-forming reactions in the cell with a variety of plant tissues[1] as well as animal tissues. Preliminary studies on respiration of plant tissue cultures aroused our interest as to the presence and function of such cytoplasmic particles in tissues cultivated *in vitro*, especially in relation to normal and diseased growth. No published report was observed on the isolation of functional subcellular structures from plant tissues cultivated *in vitro*. Therefore, experiments were made to separate cytoplasmic particles from normal and crown gall tomato tissue cultures, and to characterize their biochemical significance.

The tissue cultures, originally isolated, respectively, from normal stem and from crown gall on the stem of a tomato plant, had been cultivated for 5 years on a solid, modified White's medium[2] supplemented with 150 ml. of coconut milk per litre (*D* medium)[3]. Cytoplasmic particles were isolated by grinding the tissue in a solution containing 0·5 *M* mannitol, 0·005 *M* cysteine, 0·02 *M* dihydrogen potassium phosphate and sufficient *tris*-hydroxymethylaminomethane to bring the *p*H to 7·4, followed by a differential centrifugation. After the removal of cell debris at 500 *g* for 10 min. the active particles were sedimented at 5,000 *g* for 10 min. The particles recovered were spherical to oval bodies (0·5–2μ long axis) and showed a positive reaction to janus green *B* staining.

Oxygen consumption was measured with an oxygen electrode apparatus which consisted of a rotating platinum electrode in a cell designed for quantitative measurement of dissolved oxygen.

The particulate preparations catalyzed the oxidation by molecular oxygen of succinate, α-ketoglutarate, malate, citrate, glutamate, the reduced diphosphopyridine nucleotide and the reduced triphosphopyridine nucleotide. The oxidation of these substrates was strongly inhibited by the addition of cyanide or antimycin *A*. With most of the substrates tested, the addition of a phosphate acceptor, that is, adenosine diphosphate or a mixture of adenosine triphosphate, glucose and hexokinase, doubled the rate of oxidation (Table 1). This suggested that phosphorylation was coupled with the oxidation. This was verified by determining phosphate uptake isotopically[4] during the oxidation process. (The uptake of phosphate labelled with phosphorus-32 was measured by the increase in radioactivity in the organic phosphate fraction of the reaction mixture.) The moles of inorganic orthophosphate esterified per atom of oxygen consumed (P/O ratio) at 25°C. with various substrates are shown in Table 1.

Substitution of sucrose for mannitol in the grinding medium gave lower P/O ratios; mannitol was tested at the suggestion of W. D. Bonner, jun., Earlier, mannitol was used successfully for the preparation of mitochondria from certain plants[5], and one of us (B. H.) has used mannitol for preparing highly active mitochondria from rat liver. It appeared important

Table 1. OXIDATIVE PHOSPHORYLATION BY CYTOPLASMIC PARTICLES FROM NORMAL TOMATO TISSUE CULTURES*

Substrate	Oxygen consumption (mμatom/min./mgm.N)	P/O Ratio	Respiratory control†
Citrate	368	1·4	2·0
α-Ketoglutarate	350	1·5	1·4
Succinate	333	1·1	2·2
Malate	163	1·0	1·9
Glutamate	350	1·3	2·0

* The reaction mixture contained enzyme (234 μgm.N/ml.), substrate (2·5 μmoles/ml.), orthophosphate (5 μmoles/ml.), ethylenediamine tetraacetic acid (0·5 μmole/ml.), adenosine triphosphate (1 μmole/ml.), sufficient glucose and hexokinase, potassium chloride (20 μmoles/ml.), mannitol (0·25 *M*), and diphosphopyridine nucleotide (0·6 μmole/ml.). The last was omitted for succinate oxidation. The *p*H of the reaction mixture was 7·4.

† Ratio of rate of oxidation in the presence of the mixture of adenosine triphosphate, glucose and hexokinase to rate of oxidation in the absence of this mixture.

to maintain the *p*H slightly above neutrality (*p*H 7·0 to 7·4) throughout the isolation process to obtain active preparation with high P/O ratios. The addition of ethylenediamine tetraacetic acid did not affect the P/O ratio and respiratory activity (Q_{O_2}) of the preparation. There was little variation in the P/O ratios of the cytoplasmic particles obtained from tissues cultured for various periods up to 4 weeks after transfer. The yield and respiratory activity of the preparation, however, decreased as the cultures became older.

The cytoplasmic particles from cultures of normal and crown gall tomato tissues were qualitatively similar, but were quantitatively different in their respiratory responses and ability to support oxidative phosphorylation. Full details on this will be published later.

The results presented here indicate that tomato tissues cultivated *in vitro* contain cytoplasmic particles with oxidative and phosphorylative capacities characteristic of mitochondria.

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[1] Hackett, D. P., 'Intern. Rev. Cytol.', **4**, 143 (1955).
[2] White, P. R., 'Ann. Rev. Biochem.', **11**, 615 (1942).
[3] Hildebrandt, A. C., *Proc. U.S. Nat. Acad. Sci.*, **44**, 353 (1958).
[4] Hagihara, B., and Lardy, H. A., *J. Biol. Chem.* (in the press).
[5] Laties, G. G., *Plant Physiol.*, **28**, 557 (1953). Brummond, D. O., and Burris, R. H., *J. Biol. Chem.*, **209**, 755 (1954).

PHYSIOLOGY

An Antimineralocorticoid Action of some Saluretic Sulphonamides

It has previously been reported[1,2] that chlorothiazide and its saturated derivative, hydrochlorothiazide, have a substantial and sustained antidiuretic effect in diabetes insipidus, both experimental and clinical. The antidiuretic activity of unit weight of hydrochlorothiazide, and of its trifluoromethyl and benzyltrifluoromethyl analogues (hydrofluomethiazide and *FT*81), is many times greater than that of chlorothiazide.

Further investigation has shown:

(1) The antidiuretic property is shared by two halogenated sulphonamides ('Hygroton' and *G* 32359) in which the halogen and sulphamyl group are in the *ortho* position, but which otherwise bear little structural resemblance to chlorothiazide. The chemical names of these compounds and their approximate antidiuretic potency relative to that of chlorothiazide are given in Table 1.

(2) All the compounds which have shown antidiuretic activity are also saluretic, although their capacity to cause salt excretion has not varied at all closely with their antidiuretic potency.

Table 1

Chemical names	Common name	Antidiuretic potency (x Chlorothiazide)
6-trifluoromethyl-7-sulphamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide	Hydrofluomethiazide	50
6-chloro-7-sulphamyl-3,4-dihydro-1,2,4-benzothiadiazine-1,1-dioxide	Hydrochlorothiazide	50
6-chloro-7-sulphamyl-1,2,4-benzothiadiazine-1,1-dioxide	Chlorothiazide	1
1-oxo-3-(3′-sulphamoyl-4′-chloro-phenyl)-3-hydroxy-isoindoline	'Hygroton'	50
4′chloro-3′,4-disulphamoyl-benzophenone	*G* 32359	5
1,3-disulphamyl-4,5-dichloro-benzene	Dichlorphenamide	1
2-acetylamino-1,3,4-thiadiazole-5-sulphonamide	Acetazolamide	Nil
5-acetylimino-4 methyl-Δ 2-1,3,4-thiadiazoline-2-sulphonamide	Neptazane	Nil

(3) Two sulphonamides which share a common tubular excretory pathway with chlorothiazide, but are not saluretic (the deschlor- and methyl-sulphone analogues of chlorothiazide), had a greatly diminished antidiuretic action. Moreover, probenecid, which interferes with the tubular secretion of chlorothiazide did not reduce its antidiuretic activity.

(4) There was no qualitative difference between the antidiuretic actions of any of the effective compounds.

(5) Of three potent carbonic anhydrase inhibitors (acetazolamide, dichlorphenamide and neptazane) only dichlorphenamide which has the structural configuration mentioned above was antidiuretic. This latter compound, like the chlorothiazide drugs, and unlike acetazolamide, causes an increase in the urinary excretion of chloride as well as of bicarbonate.

It was concluded that neither the route of tubular secretion nor the carbonic anhydrase inhibitory action of chlorothiazide was the basis of its antidiuretic property. It followed that this property was related less to the global saluretic activity of the drug than to a restricted part of the action on sodium transport. Some of our recent experimental findings, listed below, suggest that the antidiuresis produced by chlorothiazide and similar drugs depends partly upon blocking the action at the kidney of the mineralocorticoid hormone of the adrenal cortex, and that the sulphonamides do this more effectively than any 'antialdosterones' previously tested.

Evidence of inhibition of mineralocorticoid action on the kidney

(1) In a series of rats with experimental diabetes insipidus the reduction of urine output which followed maximally effective doses of hydrochlorothiazide was closely duplicated by the effect of subsequent adrenalectomy. After adrenalectomy no further antidiuretic action could be induced by the drug. Further, hydrochlorothiazide had no saluretic action in adrenalectomized rats.

(2) Little expansion of the urinary output of the adrenalectomized diabetic rat followed daily injection of 2·5 mgm. cortisone; but the full pre-operative polyuria could rapidly be restored by giving cortexone in similar doses. This action of cortexone was completely blocked by hydrochlorothiazide.

(3) The daily injection of 2·5 mgm. of cortexone into non-adrenalectomized diabetic rats approximately doubled their urinary volume within one to two days. No expansion of urinary volume was produced by cortexone, even in doses twice as large as this, when it was given together with hydrochlorothiazide (Fig. 1).

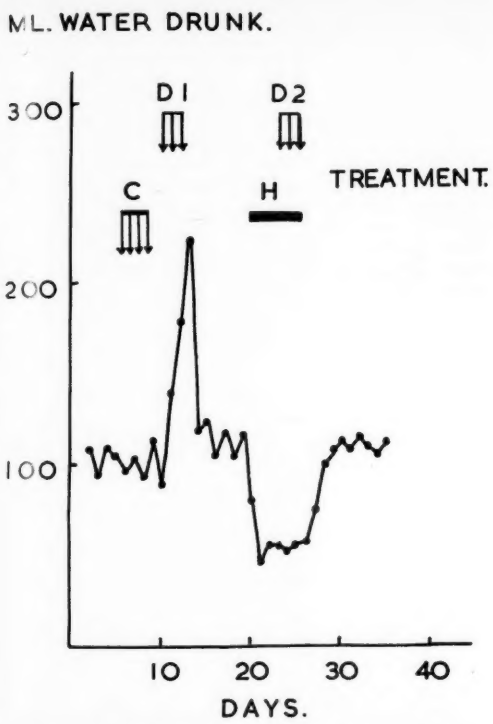

Fig. 1. Variations in the daily water intake of a rat with diabetes insipidus caused by the daily injection of 2·5 mgm. of cortisone (*C*), 2·5 mgm. of cortexone (*D*1), the oral ingestion of 32 mgm./kgm. of hydrochlorothiazide (*H*), and the injection of 5 mgm. of cortexone during hydrochlorothiazide treatment (*D*2).

(4) More prolonged treatment of normal rats with cortexone causes a diabetes insipidus like condition. This form of polyuria, like true diabetes insipidus, was approximately halved in severity, but not completely abolished, by hydrochlorothiazide.

(5) Two known antialdosterones, the spirolactones *SC* 8019 and *SC* 9420, were antidiuretic in experimental diabetes insipidus of hypothalamic origin, but their maximum effect was substantially less than that achieved by hydrochlorothiazide. Moreover, the administration of 2·5 mgm. a day of cortexone with the spirolactone overcame the antidiuretic action of the latter.

After adrenalectomy, as is well known, the kidney is unable to conserve sodium normally. Much less chloride than sodium is lost in the urine—in the rat about a third as much[3], and considerable amounts of bicarbonate are excreted. For this reason it is customary to give sodium bicarbonate along with sodium chloride to sustain adrenalectomized animals[4,5]. Cortexone corrects the disorder of electrolyte conservation.

Adrenal insufficiency also results in inability to excrete a dilute urine[6] although this defect has been attributed to unopposed or excessive action of the pituitary antidiuretic hormone, and it is corrected by cortisone rather than cortexone[7]. The present work indicates, however, that in the absence of antidiuretic hormone cortexone has a more powerful action than cortisone in diluting the urine. It seems of particular significance, therefore, that the sulphonamides which oppose this dilution of the urine, and counteract the action of cortexone, are those which normally cause the excretion of chloride as well as bicarbonate. Neither organic mercurials, which lead to an increased excretion of chloride alone, nor carbonic anhydrase inhibitors which increase the output of bicarbonate, have an antidiuretic action.

Reasons have previously been given[1,2] for believing that the antidiuretic sulphonamides inhibit that part of the tubular reabsorption of sodium which is associated with the production of osmotically free water. The present results suggest that the same part of the tubule is the principal site of action of mineralocorticoids.

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[1] Crawford, J. D., and Kennedy, G. C., *Nature*, **183**, 891 (1959).
[2] Kennedy, G. C., and Crawford, J. D., *Lancet*, *i*, 866 (1959).
[3] Buell, M. V., and Turner, E., *Amer. J. Physiol.*, **134**, 225 (1941).
[4] Harrop, G. A., Soffer, L. J., Nicholson, W. M., and Strauss, M., *J. Exp. Med.*, **61**, 839 (1934).
[5] Allers, W. D., *Proc. Mayo Clin.*, **10**, 406 (1935).
[6] Robinson, F. J., Power, M. H., and Kepler, E. J., *Proc. Mayo Clin.*, **16**, 577 (1941).
[7] Gaunt, R., Birnie, J. H., and Eversole, W. J., *Physiol. Rev.*, **29**, 281 (1949).

Eye Movement Recording

Recent work on the time course of eye movements has led to the development of a new technique of measurement. This was required to deal, in addition to the normal static situation, with conditions where space and the rigid laboratory bench are absent; for example, in a rotating chair and the human centrifuge. A wide range of sensitivity, simplicity and robust construction were considered most important, and since recordings could only be made via a slip ring system, an electrical output signal was essential. The instrument finds additional applications in circumstances where it is advantageous to keep to a minimum the loading of a deflected member, the movement of which is to be recorded.

Several elegant optical methods using the contact lens have been evolved by others in this field[1,2], but for reasons mainly connected with space and recording difficulties they are unsuitable for our applications. The present system using a contact lens follows that of Fender[3], except that his plastic stalk and mirror have been replaced by a miniature electric lamp and an aluminium tube; the tube, lamp and its connecting leads weighing less than 0·15 gm. The principle is illustrated in Fig. 1. Attached to the contact lens *A*, a lamp *C*, approximating a point source, is arranged to illuminate two optical edges *D* and *F*; a photocell *H*, positioned so that its cathode intercepts light

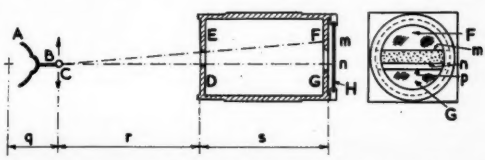

Fig. 1. Arrangement of the optical system of the photoelectric recorder

passed by both D and F, gives therefore, an output which is a linear function of the translation of C. Edges E and H serve only to exclude stray light.

The equipment is simple, contains no precision components and is capable of driving conventional recorders with no electronics other than a power supply and impedance matching device if required; in addition, the overall sensitivity and band-width is limited only by the available recorder and the resonance of the stalk B. Using a 150 cycle mirror-galvanometer, sensitivities of the order of 60 cm./deg. with a dynamic range of 50db. are practicable—these figures could be considerably exceeded but such gains are unlikely to be of value unless extreme mechanical rigidity can be secured. In Fig. 2 is shown a fast eye movement recorded by means of this apparatus.

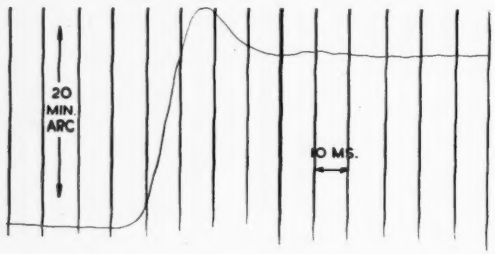

Fig. 2. Recording of a typical fast eye movement

Preliminary experimental results obtained so far indicate that: (*a*) Optokinetic or vestibular stimulation may cause nystagmus of a very small amplitude – only a few minutes of arc. (*b*) Ocular overshoot accounts for a considerable proportion of the total deflexion for small fast angular movements or flicks, but as the angle increases the overshoot tends to remain constant and fails to exhibit the characteristics to be expected of a simple physical system. (*c*) Peak velocities of fast movements are a function of the angle through which the eye is deflected. Ginsborg[4] using a contact lens and mirror, and Westheimer[5] using corneal reflexion, have also reported this relationship.

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[1] Ditchburn, R. W., and Ginsborg, B. L., *J. Physiol.*, **119**, 1 (1953).
[2] Ratcliff, F., and Riggs, L. A., *J. Exp. Physchol.*, **40**, 687 (1950).
[3] Fender, D. H., *Brit. J. Ophthal.*, **39**, 65 (1955).
[4] Ginsborg, B. L., *Brit. J. Ophthal.*, **37**, 12, 746 (1953).
[5] Westheimer, G., *Amer. Med. Assoc. Arch. Ophthal.*, **52**, 710 (1954).

Action Potentials from Single Nodes of Ranvier in Potassium-rich Solutions

Recent experiments have shown, that abnormal action potentials can be produced in (sodium-free) potassium-rich solutions[1]. In order to get more information on the ions which cross the membrane during this type of activity, the relation between the potassium concentration and the height of the action potential was examined.

Potentials from single nodes of myelinated nerve fibres (*Rana esculenta*) were recorded with the air-gap method described by Tasaki and Frank[2] (positive feedback, see also Lüttgau[3]). With this technique up to 95 per cent of the absolute potential can be obtained with outside electrodes. (Absolute resting potential of summer frogs: 56–63 mV.; ref. 4). Ringer's solution at the node under investigation (N_2) could be replaced, within several seconds, by the test solutions containing different concentrations of potassium chloride. (The test solutions were made isotonic by the addition of choline chloride.) Since potassium depolarizes the membrane, it was always necessary to repolarize by an externally applied potential between N_1 and N_2 (Fig. 1). Owing to the dependence of the potassium permeability on the membrane potential[5] the latter could not be kept at the level of the original resting potential. The externally applied compensating potential was therefore adjusted to bring the membrane potential to a new stable value (30–40 mV. above the resting potential) with a low permeability for potassium.

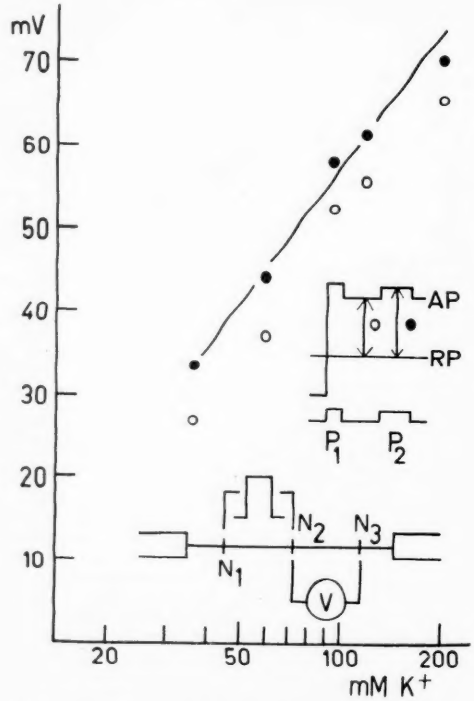

Fig. 1. Effect of change in K-concentration. Ordinates: circles, potential difference between resting potential in normal Ringer and height of action potential. Dots, same potential difference plus deflection produced by the superimposed impulse P_2 (see inset). Abscissæ: K-concentration at node 2 (inset) on logarithmic scale. Potential of 50 (36 mM K^+), 80 (59 mM K^+), and 100 mV. (94–200 mM K^+) were added between N_1 and N_3 to bring the membrane potential 30–40 mV. above the resting value. Node N_1 and N_3 were narcotized by cocain-Ringer. Each dot and circle is mean of three measurements. Corrections for junction potentials were not introduced.

The action potentials were evoked by cathodal impulses of 5 msec. duration superimposed on the remaining anelectrotonus. A typical potential, which obeys the all-or-nothing law and which lasts from 10 msec. to several seconds, starts with a sharp upstroke (in 116 mM K^+: maximum rate of rise ~100V./sec; normal value: ~500V./sec. Chronaxy 1–2 msec., normal value 0·1–0·3 msec.); it then decreases slowly ('plateau') until it reaches an unstable value, from which it returns by a quick jump to the initial position. The membrane resistance, measured as the height of the superimposed electrotonic potential P_2, increases during the plateau.

Fig. 1 shows the effect of different potassium concentrations at node 2 on the height of the potential.

The circles represent the potential difference between the original resting potential and the maximum height of the action potential after the end of the stimulus (see inset). In order to get some information about the influence of the repolarization current on the height of the potential, a cathodal impulse (P_2) of the same strength as the anelectrotonus was superimposed on the membrane during excitation, and the deflection was added to the action potential (dots). These values correspond to the membrane potentials before repolarization. The straight-line graph has a slope of 55 mV. per tenfold change of potassium concentration.

These results are in line with the following assumptions: (1) the abnormal action potentials arise from an explosive increase of the potassium permeability and an influx of potassium down the electrochemical gradient; (2) the slow repolarization to the unstable potential is due to 'inactivation' of the 'potassium-carrying-system'.

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[1] Müller, P., *J. Gen. Physiol.*, **42**, 137 (1958).
[2] Tasaki, I., and Frank, K., *Amer. J. Physiol.*, **182**, 572 (1955).
[3] Lüttgau, H. C., *Experientia*, **12**, 482 (1956).
[4] Frankenhaeuser, B., *J. Physiol.*, **135**, 550 (1957).
[5] Hodgkin, A. L., and Huxley, A. F., *J. Physiol.*, **116**, 473 (1952).

Does an Increase in the Tension in a Ligament elicit Reflex Muscular Responses?

FOR a long time articular ligaments have not been thought of as purely mechanical checks to abnormal movements of a joint; instead it has been assumed that one of their most important functions is to respond to increased tension by initiating a reflex contraction of the muscles capable of opposing the abnormal movement. This opinion has been expressed by several authors (first by Partridge[1]) and appears in orthopædic and traumatological text-books. A survey of the literature shows, however, that this teleologically attractive hypothesis has not been confirmed by experiments. In order to test the validity of the hypothesis, an investigation was begun on the cat using the medial collateral ligament of the knee joint.

A method was developed for producing considerable increase in the tension in the ligament without damaging its innervation during the preparatory dissection. The method involved loading the ligament transversally to the line of the leg by means of a thread passed round the ligament's central part via the bursa lying between the ligament and the tibial tendon of semimembranosus. That this method did not damage the ligament's innervation was shown: (*a*) by recording action potentials in the medial and posterior articular nerves on increasing the tension in the ligament; (*b*) by probing different parts of the ligament with a glass rod while recording action potentials in the medial and posterior nerves; (*c*) by using a dissection microscope to follow the course of the branches of these nerves with reference to the ligament.

By recording action potentials in the medial and posterior nerves it could be shown that a considerable afferent discharge was as a rule elicited on increasing the tension in the ligament. A study of whether this discharge induced motor effects or not constituted one way of solving the problem outlined above. Such a study (in collaboration with S. Andersson) was done using three different methods: myography, electromyography and monosynaptic testing. Successful experiments were performed on forty-nine decerebrate cats. All the muscles the contraction of which can relax the medial collateral ligament were tested. With none of the methods and in none of the muscles were reflex muscular responses obtained ascribable to receptors in the ligament. On the other hand, strong reflex muscular responses could easily be obtained by gentle mechanical stimulation of extra-ligamentary receptors within the ventromedial region of the joint. Several authors[2–5] have previously demonstrated reflex muscular responses elicited from joint receptors in the cat. In their investigations, however, they did not show to what extent ligamentary or extra-ligamentary receptors were responsible for the effect.

The supposed role of ligamentary receptors in providing reflex muscular protection for joints has been investigated on man as well (in collaboration with I. Petersén). The medial collateral ligament of the knee joint was again used. No form of anæsthetic was given. The increase in the tension in the ligament was produced in a natural way by forcibly trying to abduct the joint. The motor effect was studied by electromyography, and muscles capable of opposing the increase in the tension in the ligament were tested (sartorius, semimembranosus and vastus medialis). In thirty-five subjects (forty-three normal knee joints) no contraction was detected in the muscles mentioned during the attempted abduction, even though the medial collateral ligament was loaded with 30–40 kp. No pain was experienced on increasing the tension in the ligament.

The experiments on decerebrate cats and on unanæsthetized man complement each other. Taken together, the results suggest that under normal conditions tension recording receptors of the medial collateral ligament of the knee joint do not play any significant part in the release of a reflex muscular protection for the joint.

It appears that under pathological conditions other factors need to be considered. Thus it is known that the activity of thigh muscles may be influenced by injury to the medial collateral ligament of the knee joint. A series of experiments was started to study these reflex muscular effects by electro-physiological techniques. Ten cases have so far been investigated by electromyography. They all had the clinical diagnosis of mild injury to the medial collateral ligament with unimpaired stability of the joint. In each case, on attempted abduction of the joint, activity was evoked in sartorius (Fig. 1 *A*). In each of 6 cases where semimembranosus was tested this muscle gave a response too. In vastus medialis, on the other hand, such a response was observed in only one case out of 9. During the attempted abduction

Fig. 1. Clinical diagnosis: Partial rupture of the medial collateral ligament at the upper insertion. Electromyograph of the sartorius. *A*, response during attempted abduction of the joint. Pain was then experienced at the point *P* in the diagram. *B*, no response during attempted abduction after the injection of 2 ml. 0·5 per cent 'Carbocain' (Bofors) at the point *P*.

the subjects experienced pain at the point of 'partial rupture' of the ligament. In 5 subjects a small amount of anæsthetic was then injected at this point. Following the injection, renewed attempted abduction did not produce any muscular responses (Fig. 1 *B*). It seems likely that the reflex motor effects obtained prior to anæsthetization were due to the activation of pain receptors.

Detailed accounts of these experiments on cats and man will be published elsewhere.

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[1] Partridge, E. J., *J. Anat.*, **58**, 346 (1924).
[2] Gardner, E., *Amer. J. Physiol.*, **161**, 133 (1950).
[3] McCouch, I. D., Deering, I. D., and Ling, T. H., *J. Neurophysiol.*, **14**, 191 (1951).
[4] Skoglund, S., *Acta Physiol. Scand.*, **36**, Suppl. 124 (1956).
[5] Cohen, L. A., and Cohen, M. L., *Amer. J. Physiol.*, **184**, 433 (1956).

Peptides with Oxytocic and Pressor Activity obtained from Acidified Rat Serum

Incubation at 38° produces an increase in oxytocic activity in the acidified serum of normal rats (Croxatto, H. B., and Croxatto, H., unpublished work). Earlier studies have revealed that pepsitensin-like substances can be obtained from hypertensinogen under similar conditions[1]. This communication describes experiments carried out to determine some of the properties of the substance (or substances) extracted from acidified and incubated rat serum.

Blood was drawn without anticoagulant by aortic puncture from rats anæsthetized with ether or bromethol. It was centrifuged at 0° and the serum was acidified to *p*H 3·8 with hydrochloric acid. The period of incubation at 38° varied from 1 to 30 hr. The proteins were then precipitated with nine volumes of absolute ethanol. The supernatant was evaporated at low temperature and the residue suspended in distilled water for biological assay. Further purification was achieved by removing the lipids with petroleum ether. The aqueous solution remaining was again evaporated and the residue dissolved in glacial acetic acid. Ten volumes of a mixture of petroleum ether and absolute ethanol were then added. The residue was dissolved in distilled water to half the original volume of the serum. The solution was tested on the isolated rat uterus suspended in Tyrode solution containing atropine and dibenamine. Extracts from 1 ml. rat serum showed an oxytocic effect similar to that of 10–50 mU. oxytocin and contracted the isolated rat ileum like 0·5–1 U. of pepsitensin or hypertensin I.

The solubility characteristics and the effect of proteolytic enzymes on the unknown oxytocic substance suggest a polypeptide structure. It is rapidly destroyed by chymotrypsin and carboxypeptidase and partially destroyed by pepsin and trypsin (30–50 per cent). Under similar conditions, trypsin completely inactivates vasopressin and hypertensin I and II. That the substance is different from oxytocin is demonstrated by its lack of milk ejection activity in the lactating rabbit, its resistance to the action of sodium thioglycollate and by its different reaction to carboxypeptidase, pepsin and trypsin.

The peptide differs also from the neurohypophysial hormones in its vasopressor effects: whereas it produced a striking increase in the blood pressure of rats nephrectomized 12–24 hr. previously, no effect or a slight fall was seen in intact anæsthetized rats.

The progressive liberation of this substance is probably due to an enzymatic action at 38–40° at an optimum *p*H of approximately 3·8. Incubation for 24 hr. liberates practically all oxytocic and vasopressor activity from acidified rat serum. Little or no active substance was obtained when serum was heated for 30 min. at 58°C. before acidification and incubation. Incubation for a few hours at 38° at normal *p*H was also ineffective. The enzyme active in acidified serum is not renin, since serum from nephrectomized animals yields higher activity. A gastric origin of the enzyme can also be excluded because similar quantities of active peptide can be obtained from the serum of gastrectomized animals. The pressor and oxytocic activities are rapidly destroyed when purified samples of the peptide are incubated at neutral *p*H in the presence of small volumes of blood serum.

The polypeptide liberated by the simple procedure of acidification and incubation is different from pepsitensin and hypertensin I and II, but judging from its action on the rat uterus and guinea pig ileum it resembles bradykinin[2]. However, bradykinin has a strong vasodepressor effect in several mammalian species and is less active on the hen's rectal cæcum. Further investigations will be necessary to ascertain whether the peptide liberated under the above described conditions is related to the oxytocic substance described by Hawker[3] and to peptides obtained by the action of pepsin on blood serum. The substance is now being purified to obtain information on the possibility that it has a physiological function and plays a part in the pathogenesis of renoprival hypertension.

A full account of these experiments will be published elsewhere.

We wish to thank the Gildemeister Foundation for the financial aid given us in the work here reported. We are indebted to Dr. L. T. Skeggs for his generous supply of natural hypertensin I and synthetic hypertensin II.

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[1] Alonso, O., Croxatto, R., and Croxatto, H., *Proc. Soc. Exp. Biol. Med.*, **52**, 61 (1943).
[2] Rocha e Silva, N., 'Polypeptides which stimulate Plain Muscle', edit. by Gaddum, J. H. (E. & S. Livingstone, Ltd., Edinburgh, 1955).
[3] Hawker, R. W., and Robertson, P. A., *Endocrin.*, **63**, 242 (1958).

VIROLOGY

Antiviral Chemotherapeutic Activity of Isatin β-thiosemicarbazone in Mice infected with Rabbit-pox Virus

Isatin β-thiosemicarbazone has been found to have a very powerful chemotherapeutic effect in mice infected intracerebrally with the *IHD* strain of neurotropic vaccinia virus[1], and will give complete protection against death after infection with doses of virus as high as 100,000 *LD*50[2]. The compound is inactive

against ectromelia infection in mice, however[3], and it is therefore of interest to investigate its action against other members of the pox group. The present work was carried out with the Utrecht strain of rabbit-pox virus[4], presented by Dr. E. A. Boulter, of the Microbiological Research Establishment, Porton. In view of the similarity of this virus to vaccinia and the uncertainty as to its origin, a preliminary investigation was undertaken to ascertain whether it does in fact differ in biological characteristics from the *IHD* strain of neurovaccinia used in previous work. That a distinction does exist was shown by the failure of rabbit-pox virus to kill mice of weight greater than 15 gm. when inoculated intracerebrally. Differences were observed also in tissue culture. Propagation of both viruses in the cell strain *ERK* I[5] yielded fluids of high titre; but whereas the cytopathic effect of neurovaccinia consisted of granularity and swelling of individual cells followed by rounding and detachment from the glass, that of rabbit-pox virus manifested itself as the formation of syncytial sheets of cells which later became filled with large vacuoles.

The tests of chemotherapeutic activity of isatin thiosemicarbazone were carried out by the method described previously[2]. A titration of a suspension of mouse brain infected with rabbit-pox virus was carried out in parallel, two groups of 6 mice being infected intracerebrally with each of 5 decimal dilutions of virus. One series of mice was injected subcutaneously twice daily with 2 mgm. of isatin β-thiosemicarbazone in aqueous suspension, the first dose being given 4 hr. after infection, to a total of 9 doses, and the second series of mice was left untreated as a control. Twenty-seven of the 30 control mice, which had received a virus dose ranging from 10 to 100,000 *LD*50, died after periods ranging from 3 to 7½ days. The treated mice all survived, and the compound thus gave complete protection against infection with 100,000 *LD*50 of virus. After 13 days the treated mice were given an intracerebral challenge with about 1,000 *LD*50 of rabbit-pox virus; all survived, whereas all of four control animals infected with the same dose after challenging the treated animals died after 3 days. Mice which survive rabbit-pox infection as the result of treatment with isatin β-thiosemicarbazone therefore develop a high degree of immunity. Intracerebral challenge with neurotropic vaccinia virus was carried out 7 days later; all the treated mice survived and were discarded in apparent health 57 days after the beginning of the experiment, whereas 4 control mice inoculated with the challenge material at the same time all died after 3–4 days. Essentially similar results were obtained in further experiments in which over 200 mice were used.

The results so far obtained therefore show that the pox viruses fall into two groups with regard to their susceptibility to isatin β-thiosemicarbazone; vaccinia and rabbit-pox are highly sensitive, whereas ectromelia is resistant. The behaviour of other members of the pox virus group is now being investigated.

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[1] Thompson, R. L., Minton, S. A., Officer, J. E., and Hitchings, G. H. *J. Immunol.*, **70**, 229 (1953).
[2] Bauer, D. J., *Brit. J. Exp. Path.*, **36**, 105 (1955).
[3] Lock, M., *Z. Hyg.*, **143**, 480 (1957).
[4] Jansen, J., *Zentr. Bakteriol. Parasitenk.*, I, **148**, 65 (1941).
[5] Westwood, J. C. N., MacPherson, I. A., and Titmuss, D. M. J., *Brit. J. Exp. Path.*, **38**, 138 (1957).

Effect of Concentrated Urea Solutions on the Infectivity and Hæmagglutinin of Vaccinia Virus

During preliminary experiments attempting to extract deoxyribonucleic acid from vaccinia virus by various physical and chemical means, we incidentally observed that after exposure to 8 *M* urea the infectious properties of the virus were rapidly lost in the cold, whereas the hæmagglutinin titre was not reduced even after several hours at 37° in the presence of the reagent. These findings seemed sufficiently interesting to prompt the further investigations described in this communication.

In the first three experiments recorded in Table 1, the infectious particles from two equal volumes of crude vaccinia virus suspension (strain Levaditi adapted to the chick embryo chorioallantois) were deposited by centrifugation at 30,000 *g*. One pellet was resuspended in 8 *M* urea (*p*H 7·2) with the aid of a glass homogenizer, and the other pellet was similarly resuspended in an equal volume of buffered physiological saline. In experiments 6 and 7, virus was diluted directly in the urea solution, or in buffered saline for the controls, without preliminary centrifugation. After the various times and treatments shown in Table 1, the virus preparations were diluted in series and inoculated into groups of 12-day chick embryos. Pock counts were made after incubation for 48 hr.

Table 1. Effect of 8 *M* urea on vaccinia virus infectivity

Exp. No.	Conditions	Infectivity Titre* Urea	Control
2	Centrifuged†; resuspended 37°−30 min.	$<10^3$	2×10^6
3	Centrifuged; resuspended 37°−5 min.; dialyzed†	$<10^2$	5×10^6
5	Centrifuged; resuspended 10°−2 min.; dialyzed†	1×10^4	2×10^6
6	Diluted in urea; dialyzed† immediately	$<10^2$	8×10^6
7	Diluted in urea; stood 20°−10 min.	5×10^3	3×10^7

* Pock-forming units/0·1 ml.

† Treated and control virus dialyzed 5 hours against running water at 4°.

The results recorded in Table 1 show that a highly significant reduction in infectivity occurs after standing in 8 *M* urea at 37°, and also after resuspending as rapidly as possible in 8 *M* urea at 10°, followed immediately by dialysis at 4° (experiments 2, 3 and 5). Extensive inactivation also occurs when virus is diluted 1:10 directly in urea, and then either dialyzed in the cold as rapidly as possible, or inoculated after standing at 20° for 10 min. (experiments 6 and 7). Other experiments have shown a less than 1 log reduction in titre when virus is exposed to 5 or 6 *M* urea for 10 min. at 20°. Inactivation is apparently irreversible since it was found that the titre fell further after prolonged dialysis.

The heat stability of the hæmagglutinin in the presence of 8 *M* urea was next examined. It was found that the titre remained undiminished, or even increased slightly, after heating in urea at 50° or 70° for up to 2 hr.; and at 60° the results illustrated in Fig. 1 were obtained. Virus was diluted 1:10 in 8 *M* urea *p*H 7·2, or in *M*/15 phosphate buffer *p*H 7·2, heated at 60° ±0·10, and samples removed for titration at the times shown. It will be seen that the titre of the control in buffer falls rapidly during the first 45 min., then remaining unchanged. This is in contrast to the results of Gillen, Burr, and Nagler[1], who found that the hæmagglutinin titre of undiluted crude virus was reduced by 50 per cent only after

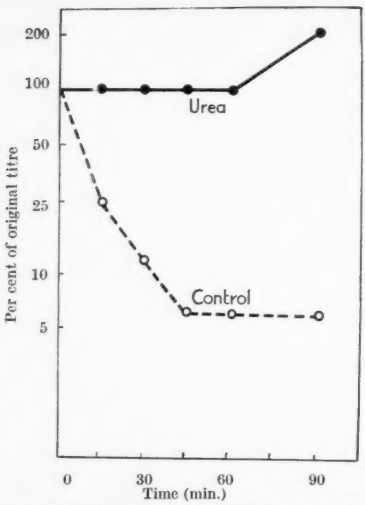

Fig. 1. Effect of 8*M* urea on the hæmagglutinin titre of Levaditi strain vaccinia virus heated at 60°. Titre at zero time 1:640

1 hr. at 70°. The titre of hæmagglutinin heated in 8 *M* urea, on the other hand, remains constant within experimental error for 1 hr. under the same conditons, and then increases slightly. This effect, which was contrary to expectation, has been consistently obtained in numerous further experiments. It is apparent, therefore, that 8 *M* urea is a protective agent against heat inactivation of the vaccinia hæmagglutinin under the conditions described. A similar effect has been observed with 5 *M* guanidine-hydrochloride It should be noted that urea alone does not agglutinate red cells at the dilutions used in our tests, and that the hæmagglutinin is still completely neutralized by specific antiserum at the end of the heating period. The hæmagglutinin of virus heated in 4 *M* sodium chloride is inactivated at the same rate as that of control virus in 0·9 per cent sodium chloride, suggesting that the protective effect is not simply due to solute concentration.

The specific action of concentrated urea solutions on proteins is believed to be due mainly to breaking of hydrogen bonds, with consequent unfolding of previously coiled structures. While it is easy to speculate that such a mechanism could account for loss of infectivity through disorientation of binding sites on the virus surface, it is difficult to explain protection against heat inactivation of the hæmagglutinin in similar terms. Control virus, diluted 1:3 or 1:10 in *p*H 7·2 phosphate buffer, becomes turbid within a few minutes at 60° or 70°, whereas virus heated in the presence of 5–8 *M* urea or 4–6 *M* guanidine in the same buffer remains perfectly clear after several hours at these temperatures. Possibly the protective effect of urea is thus due to its preventing coagulation of substances in the virus preparation, which, in the absence of urea, combine with and inactivate the hæmagglutinin. The consistent increase in hæmagglutinin titre after heating for 1–2 hr. at 60°, which is observed most strikingly with 6 *M* guanidine, is presumably due to liberation of 'soluble' hæmagglutinin units from aggregates. We have not observed any significant increase in the titre of the 'bound' hæmagglutinin[1] when elementary bodies purified by 4 cycles of differential centrifugation are heated in urea.

Vaccinia hæmagglutinin apparently contains a lipid component essential for biological activity, since in crude virus preparations it is inactivated by lecithinases present in *Cl. welchii* and cobra toxins[2], and hæmagglutinin separated from infectious virus is also inactivated by highly purified lecithinase *C*[3]. It is possible that urea also has a direct effect on lipids or lipoproteins in the hæmagglutinin and further experiments attempting to explain the mechanism of the protective effect of urea on the hæmagglutinin are in progress.

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[1] Gillen, A. L., Burr, M. M., and Nagler, F. P., *J. Immunol.*, **65**, 701 (1950).
[2] Stone, J. D., *Aust. J. Exp. Biol. Med. Sci.*, **24**, 191 (1946).
[3] McCrea, J. F. (unpublished results).

HISTOLOGY

Actual Distribution of the Number and Volume of the Islets of Langerhans in Different Size Classes in Non-diabetic Humans of Varying Ages

The equations defining the relationship between the apparent size distribution, that is, that observed in a section and the actual size distribution of spherical and ellipsoidal bodies were derived by Wicksell[1,2]. With the assumption that these bodies are evenly distributed, the relationship can be expressed in the form of Abelian integrals, which by approximation can be converted into linear equation systems adapted for practical calculation.

The biological application of these equations has proved to be of especial importance in quantitative studies of the islets of Langerhans. Thus, investigations on the rat showed that, in plotting the total islet volume in relation to the islet diameter, a symmetrical curve was obtained regardless of the age of the animals, and that this curve in the main part of its course approximated to a normal distribution[3,4,5]. The mean values and scatter in these distributions were such that a linear relationship was obtained between the logarithms of the number of islets and their diameters in those islet size classes which contained the overwhelming majority of the islets studied[5,6]. As a result of the regular arrangement of the islet tissue, it was possible to determine the total islet volume in a rat by counting only the larger islet section surfaces in a number of representative serial sections[7].

In order to investigate whether the islet tissue in man was similarly, regularly arranged, and if so what basic pattern existed, the numerical and volumetric distribution of the islets were studied in different regions of the non-diabetic human pancreas. It was in this case also possible to use the equations given by Wicksell, as the form of the islets only in exceptional cases obviously deviated from the spherical or ellipsoidal, and the requirements for randomness of the islet dispersion within the exocrine tissue were apparently well satisfied to the extent necessary, that is, for a rather small part of one single region. Pancreases from altogether 14 individuals of ages varying from newly born to 84 years old were examined. The distribution curves from every region analysed were calculated on the

basis of measurements of at least 500 islets with a mean diameter of $\geqq 46{\cdot}9\mu$. The highest number of regional analyses made from a single pancreas was 12, corresponding to size determinations of more than 6,000 islet section surfaces. By comparing fresh pancreatic tails, which had been resected during gastric operations, with pieces which had been kept at + 4°C. for up to 48 hr., it was possible to show that postmortem changes did not affect the results.

If the number of islets was plotted in relation to the islet diameter, in all ages and regions asymmetrical curves were obtained showing that the number of islets increased progressively with decreasing islet diameter. An example of this is given in Fig. 1, where the mean values for the percentage distributions in different regions are given both for a newly born infant and an adult. It should be noted that this progressive increase in the number of islets with decreasing diameter was proportionately most pronounced in those cases where larger islets were lacking, that is, for the newly born (Fig. 1). In those pancreases where the islet analyses were carried out on samples taken at narrow regular intervals, the mean distribution curves of the total islet volume and the total number of islets for all the regions can be said to reflect closely the corresponding distributions for the whole organ. It was therefore interesting to observe that the logarithm of the mean number of islets in these cases was an approximately linear function of the islet diameter in those size classes which contained the overwhelming majority of the islets studied.

While numerically the small islets strongly predominated, the main part of the total islet volume was, on the contrary, contributed by the medium-sized islets. The volumetric distribution curve of the islets was found to be symmetrical regardless of age. An example of this is shown in Fig. 2, from analyses of the same organs as in Fig. 1. In Fig. 2, however, the separate distribution curves for the head, body and tail regions are also given in addition to the mean value curves. In the few cases where the regional distribution curves showed more obvious deviation from the basic symmetrical form, this was found in analyses from adjacent parts of the head and the body of the pancreas in adults[8]. A possible explanation of why irregularities can appear in this particular area may be that it represents the fusion zone for the ventral and dorsal pancreatic primordia. If the values for the mean volumes in the different size classes for the regional analyses in a single pancreas were expressed as cumulative per cent of the total islet volume, their logarithms were grouped mainly along straight lines, showing that the symmetrical curves in the important parts of their courses, that is, with the exception of the extreme values in both directions, closely approximated to normal distributions.

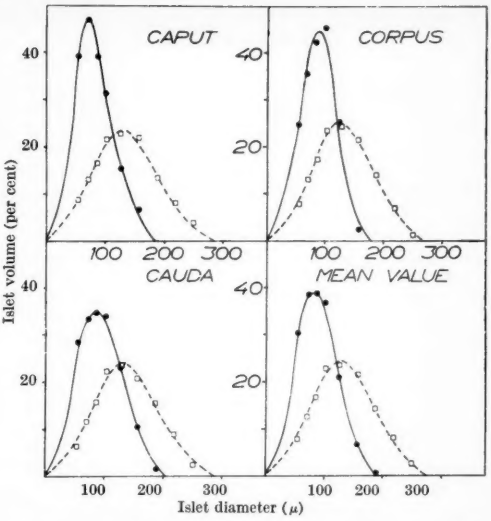

Fig. 2. Volume distribution curves in per cent for the same organs as in Fig. 1. Separate curves for the head, body and tail regions are given in addition to the mean value curves. The notation and compensation of values for variation in class width are as in Fig. 1. Note that independent of age the volume curves in all three pancreatic regions are symmetrical, but that the volume curve maximum in the adult pancreas is shifted to the right while the whole curve is significantly broader than in the newborn.

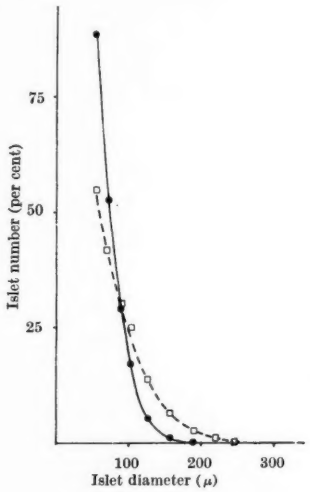

Fig. 1. An example of the percentage frequency distributions of the numbers of islets in a newborn infant and an adult. The figures which represent the mean values for the head, body and tail have been given in relation to the islet diameters expressed in μ. The values in the first four classes are plotted as double the actual values in order to compensate for the fact that these classes were only half the width of the other classes.

As in rats, the actual size distribution of the islets in non-diabetic humans was thus characterized, independent of age, by a striking regularity of its arrangement. This was evident from the fact that a simple mathematical relationship was found both for the numerical and volumetric distributions in terms of the islet diameter. These findings can be expected to be of importance for the assessment of the role of the islets of Langerhans in different types of human diabetes and should facilitate the introduction of simplified methods for the estimation of the total islet volume in man, similar to those described for the rat[7].

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[1] Wicksell, S. D., *Biometrika*, **17**, 84 (1925).
[2] Wicksell, S. D., *Biometrika*, **18**, 152 (1926).
[3] Tejning, S., *Acta Med. Scandinav.*, Suppl. 198 (1947).
[4] Hellman, B., *Acta Endocrinol.*, **31**, 91 (1959).
[5] Hellman, B., *Acta Soc. Med. Upsal*, **64**, 393 (1959).
[6] Hellman, B., *Acta Endocrinol*, **32**, 63 (1959).
[7] Hellman, B., *Acta Pathol. et Microbiol. Scandinav*, **47**, 21 (1959).
[8] Hellman, B., *Acta Soc. Med. Upsal*, **64**, 432 (1959).

Ultra-structure of the Myoneural Junction in Myasthenia Gravis

In myasthenia gravis the leading abnormality appears to be a disorder of transmission of the nerve impulse from the terminal expansions of the neural component of the end-plate across the myoneural junction to the muscle. The transmission of impulses is promptly restored by administration of anti-cholinesterasic drugs suggesting that the essential structural abnormality, if such there be, is a fine one. For this reason one must feel a reluctance to accord a primary role in the disorder of neuromuscular transmission to the florid and apparently permanent changes in the end-plates demonstrated by vital staining in myasthenic patients by Coërs and Woolf[1], Woolf *et al.*[2] and Bickerstaff and Woolf[3].

An abnormality in the ultra-structure of the synaptic region in such end-plates would, however, be more acceptable as the morphological component of this reversible disorder of transmission. We have, therefore, in our present study of the ultra-structure of the human end-plate in myasthenia and other diseases paid special attention to the synaptic region. Naturally many additional data have been accumulated at the same time, but this will be the subject of a later report when a larger number of cases have been studied and the significance of the variations in structure encountered can be more reliably assessed.

What we wish at this stage to report is: (*a*) that a compound synaptic membrance similar to that described by Robertson[4] in the chameleon lizard also occupies the synaptic troughs of human end-plates both from non-myasthenic and myasthenic patients; (*b*) that we have been unable to demonstrate until now any significant difference in the structure of the membrane in the two cases.

It will be observed that slightly better preparations were obtained from the non-myasthenic patient (suffering from a peripheral neuropathy following Wernicke's encephalopathy) and in these we are able to see not only the five layers described by Robertson, but two further layers formed by the resolving of the central dense zone into a three-layered structure, two dense layers bordering on an inner lighter layer. This innermost light zone was observed by Robertson only in the depths of the junctional folds. We have also been able to demonstrate in both patients that there appear to be occasional pores in the dense line next to axoplasm, of such a size that the vesicular structures believed to be packets of acetyl choline molecules could pass through the pore from the terminal axoplasmic expansion into the first light layer or zone. Robertson also noted in many regions fragmentation or absence of this line. He was, however, uncertain as to whether such breaks were physiologically significant discontinuities or artefacts. The borders of the pores in our preparations are such as to incline us to regard them as physiologically significant. We have also observed that the central three layers (Robertson's central dense zone) split at the mouth of a junctional fold, so that within the latter the seven-layered arrangement will continue. Our observation that Robertson's central dense zone has a light layer within it suggests that this may be an important channel within which substances may circulate both in the synaptic region and within the troughs.

While we have been unable to demonstrate any significant difference between the synaptic regions in the myasthenic and non-myasthenic patient, this must not be taken to indicate that no such difference exists, rather it provides an indication of the structures to be studied in the course of further investigations.

Fig. 1. The compound synaptic membrane in end-plates from non-myasthenic and myasthenic patients. In all the illustrations the neural aspect of the membrane is uppermost. *a*, Non-myasthenic patient—the membrane can be seen, especially to the right, to consist of 7 layers (×86,000); *b* and *c*, myasthenic patient. A membrane of at least 5 layers is demonstrated and in (*c*) a pore in the outermost layer (marked with an arrow) can be seen with axonal vesicles in its vicinity (*b* ×100,000; *c* ×37,000); *d*, diagrammatic representation of the laminal structure of the synaptic membrane showing the thickness of the layers. This is based on (*a*), and 800 A. is probably a maximum measurement, the synaptic membrane in (*b*) measuring 600 A.

It remains to add that the myasthenic patient whose end-plates are referred to here was a young woman with typical myasthenic weakness and fatiguability affecting the muscle sampled by biopsy, prior to which she had not been treated with anti-cholinesterasic drugs.

We wish to thank Dr. D. R. Humphreys for allowing us to refer to the non-myasthenic patient who was under his care.

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[1] Coërs, C., and Woolf, A. L., C. R. Cong. Médicins Aliénistes et Neurologistes, Liége, July 19–26, 1954; The Innervation of Muscle, 101 (Blackwell, Oxford, 1959).
[2] Woolf, A. L., Bagnall, H. J., Bauwens, P., and Bickerstaff, E. R., *J. Path. Bact.*, **71**, 173 (1956).
[3] Bickerstaff, E. R., and Woolf, A. L., *Brain* (in the press).
[4] Robertson, J. D., *J. Biophys. Biochem. Cytol.*, **2**, 381 (1956).

RADIOBIOLOGY

A New Approach to the Mechanism of Mutations

DATA we have gathered concerning the mechanism by which X-rays block the biosynthesis of nucleic acid led us to postulate a hypothesis on the origin of X-induced mutations, which may also explain the spontaneous mutations.

It was first observed that 12 hr.—21 days after a 700-r. whole-body irradiation was the best interval for the study of the mechanism of X-inhibition of rat spleen ribo- and deoxyribo-nucleic acid biosynthesis. Thus, between 12 hr. and 10 days, the quantities of nucleic acid remain steadily well below the normal (−50 75 per cent), while after 21 days there is a considerable increase of nucleic acid (+50 per cent) indicating intense synthesis. If phosphorus-32 is injected 12 hr. prior to killing, the diminution of relative specific activities (relative to inorganic phosphorus) are for deoxyribo- and ribo-nucleic acids respectively 55 and 40 per cent 12 hr. after irradiation, 70 and 52 per cent after 2½ days, and 52 and 20 per cent after 4 days. On the 7th day after exposure, the relative specific activity of the two nucleic acids are normal; on the 21st day, there is an augmentation of +400 per cent for deoxyribonucleic acid and +300 per cent for ribonucleic acid. Relative specific activity of free nucleotides are similar for irradiated and control animals, while there occurs a considerable increase of ribonucleotides, especially of ribonucleoside triphosphates at 12 hr., 2½ days, 4 days, 7½ days and 21 days, deoxyribonucleic acid serving as reference (Fig. 1).

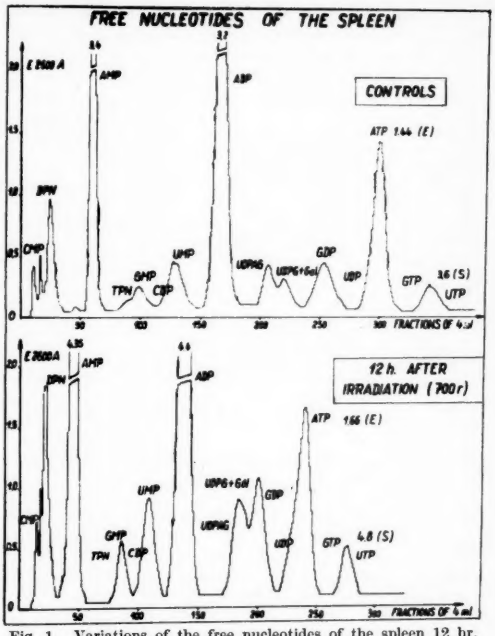

Fig. 1. Variations of the free nucleotides of the spleen 12 hr. after a 700 r. whole-body irradiation. The nucleotides are separated on formate Dowex anion exchange columns. The charts of control and irradiated animals correspond to the same amount of cellular deoxyribonucleic acid.

The technical difficulties of assay do not allow us to present strictly quantitative results for free deoxyribonucleotides and particularly deoxyribonucleoside triphosphates. However, the latter do also increase. The same phenomenon occurred in an ascites hepatoma 12 hours after whole-body irradiation of the rat (Fig. 2). These data are in agreement with the work of Errera[1], Forssberg and Klein[2], Bishop and Davidson[3], Ord and Stocken[4] who, after shorter delays and with different biological material, made observations similar to ours.

The increase of ribo- and deoxyribo-nucleoside triphosphates after irradiation, while their relative specific activity is nearly normal and while the nucleic acid biosynthesis is greatly reduced, corresponds to an accumulation of these compounds the synthesis of which persists but the polymerization of which leading to ribo- and deoxyribo-nucleic acid is partially stopped under the effect of X-rays.

It seems likely that the accumulation of ribo- and deoxyribo-nucleoside triphosphates, some of which take part in biosynthesis other than that of nucleic acid and less affected by X-rays, may lead to an abnormal molar ratio between ribonucleoside triphosphates, precursors of ribonucleic acid, and deoxyribonucleoside triphosphates, precursors of deoxyribonucleic acid. One may also conceive that an abnormal ratio of precursors produces abnormal ribo- and deoxyribo-nucleic acid, as may be inferred from the *in vitro* experiments with the enzyme of Manago and Ochoa[5] on ribonucleic acid and of Kornberg *et al.*[6,7,8] on deoxyribonucleic acid. Thus, when the biosynthesis of deoxyribonucleic acid resumes explosively from an abnormal molar ratio of precursors once the effects of irradiation on the polymerase decrease, there may occur the production of an abnormal deoxyribonucleic acid leading to mutations. In the same way, spontaneous mutations may result from metabolic activity which may draw out preferentially one type of deoxyribonucleoside triphosphate leading in this way to a modification of the normal ratio of precursors of deoxyribonucleic acid and thence to a modified deoxyribonucleic acid.

Finally, the presence of deoxyribonucleic acid viruses in the cells, with their pool of precursors: deoxyribonucleoside triphosphates, may modify the host deoxyribonucleic acid biosynthesis and facilitate

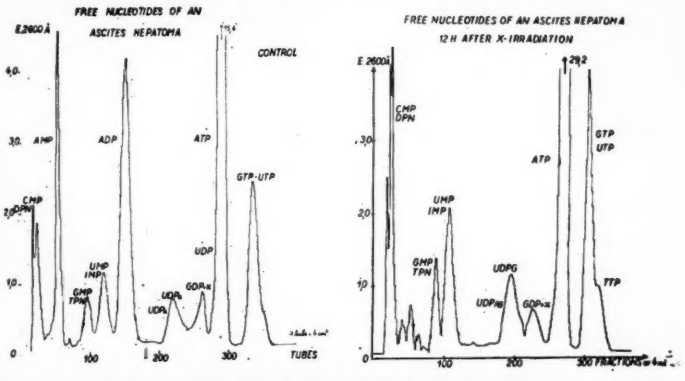

Fig. 2. Free nucleotides of an ascites hepatoma of control rats and 12 hr. after a 700 r. whole-body irradiation. The nucleotides are separated on formate Dowex anion exchange columns. There appears after irradiation a peak of thymidine triphosphate (TTP) which does not exist in controls.

the occurrence of mutations. We explain in the same way the observations that a phage-infected *E. coli* cell ceases to produce its own deoxyribonucleic acid and makes instead the acid characteristic of the infecting phage[9]. This of course is but a hypothesis, which we are now trying to confirm.

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[1] Errera, M., in Protoplasmatologia, "Handbuch der Protoplasmaforschung", **10**, 3 (Springer-Verlag, 1957).
[2] Forssberg, A., and Klein, G., *Exp. Cell. Res.*, **7**, 480 (1954).
[3] Bishop, C. W., and Davidson, J. N., *Brit. J. Radiol.*, **30**, 367 (1957).
[4] Ord, M. G., and Stocken, L. A., *Biochim. Biophys. Acta*, **29**, 201 (1958).
[5] Grunberg-Manago, M., Ortiz, P. J., and Ochoa, S., *Biochim. Biophys. Acta*, **21**, 197 (1956).
[6] Adler, J., Lehman, I. R., Bessman, M. J., Simms, E. S., and Kornberg, A., *Proc. U.S. Nat. Acad. Sci.*, **44**, 641 (1958).
[7] Lehman, I. R., Zimmerman, S. B., Adler, J., Bessman, M. J., Simms, E. S., and Kornberg, A., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1191 (1958).
[8] Bessman, M. J., Lehman, I. R., Simms, E. S., and Kornberg, A., *J. Biol., Chem.*, **233**, 171 (1958).
[9] Hershey, A. D., Dixon, J., and Chase, M., *J. Gen. Physiol.*, **33**, 777 (1952–1953).

Effect of X-Radiation on the Survival of Yeast in Dry and Wet Conditions

There is a great deal of uncertainty regarding the direct and indirect actions of ionizing radiation on cells[1,2]. The indirect effect occurs when chemically active intermediates are formed by the action of ionizing radiation on water surrounding the sensitive zones. These intermediates are capable of diffusing through the cell and of inactivating the vital molecules. The effect of direct action may be measured by irradiating dry material, in which case the energy is released directly by the ionizing radiation within the molecule which is inactivated. Several studies have been made on the survival of yeast cells irradiated in the wet condition[3,4,5], but the results observed are the combined effect of both direct and indirect actions. In order to assess the relative contributions of the two types of action, yeast cells in the dry and the wet state, but otherwise in exactly identical condition, have been irradiated with X-rays, so that from the observed difference in the radiosensitivity the contributions of the direct and indirect actions could be estimated.

The strain of yeast used was a selected strain of *Saccharomyces cerevisiae* in dry granular form (pure dry yeast, Distillers Co., Ltd., Edinburgh). About 94 per cent of the cells were single and 6 per cent in the budding condition.

The source of X-irradiation was a Picker Army Field X-ray Unit operated at 70 kV. and 3–4 m.amp. filtered by $\frac{1}{4}$ mm. aluminium. The samples were contained in a cylindrical 'Perspex' box (6·8 cm. diameter, 2·6 cm. long) which fitted tightly in the window opening, and samples were always placed at definite positions within the exposure box. The dose-rate of X-rays at the place where samples were exposed (7 cm. distance from the target) was 690 r./min. inside the 'Perspex' box. The dose-rates were determined with a Victoreen condenser r.-meter within an accuracy of $\pm 2{\cdot}5$ per cent.

For irradiation in the dry condition 10 mgm. of the dry commercial yeast granules were weighed accurately and exposed in small glass tubes of 3 mm. diameter and 3 cm. long to previously calibrated doses of X-rays. The granules for each separate dose were then dropped into 10 ml. of previously sterilized distilled water. About 15 min. later, when the granules had dissolved, the solution was shaken vigorously in order to disperse the cells evenly.

For irradiation in the liquid condition, 10 mgm. of dry yeast granules were weighed carefully and dissolved in 10 ml. of sterile distilled water. This formed the stock solution and contained about 10^7 cells per ml. After shaking vigorously, one drop of stock solution was distributed in each of several small glass capillaries containing 0·1 c.c. of sterile distilled water. These were exposed to different doses of X-radiation and then each tube was dropped in 9·9 ml. of sterile distilled water contained in glass test-tubes.

In both the cases—dry as well as wet—the number of viable cells was determined by surface plating, after necessary dilutions, on solid Sabouraud's media (peptone 1 per cent, maltose 4 per cent, agar 2·3 per cent, water 100 ml.). Both irradiation and platings were done at room temperature which varied between 28° and 30°C. The colonies were counted 36–48 hr. after plating.

For each set of experiments non-irradiated controls were kept, and with the exception of irradiation all other procedures and preparation methods were the same. The survival fraction was determined for each dose as shown in Fig. 1. Each point in Fig. 1 is the mean of at least five sets of separate experiments, two plates per point per set and therefore ten plates per dose.

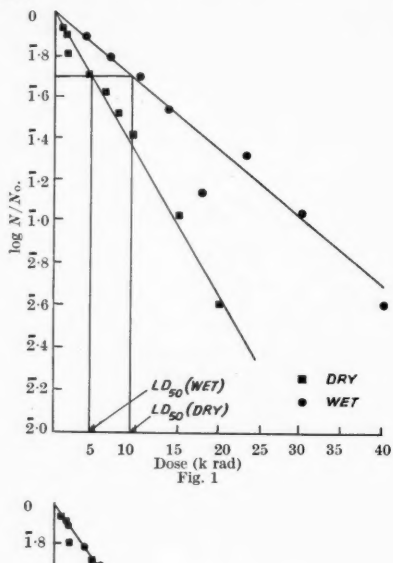

Fig. 1

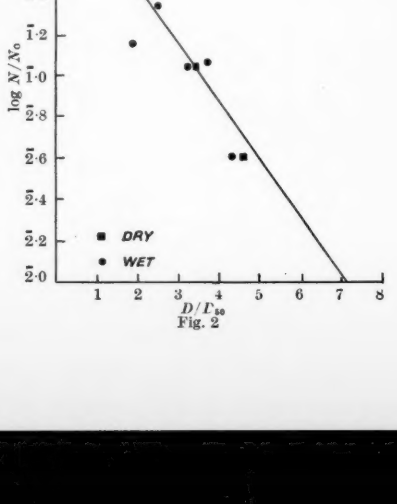

Fig. 2

Fig. 1 compares the effect of X-irradiation on yeast cells in the dry and the wet condition. The fraction surviving (N/N_0) where N is the number of cells surviving and N_0 is the total number of cells at a particular dose, has been plotted semilogarithmically against dose in kilorads.

50 per cent lethal dose (LD_{50}) in the case of yeast in the wet condition, as obtained from the graph was 4·4 kilorads. This dose compares favourably with the doses of 1·8–4 kilorads reported by other observers[3–5]. Under the same condition of irradiation but with dry yeast, LD_{50} was 9·4 kilorads. Fig. 2 shows the results plotted against D/D_{50} for both dry and wet cells. It is found that both sets fall on the same straight line, as is to be expected from the single-hit theory.

The present experiment shows conclusively that there is considerable difference between the irradiation of dry cells and those held in suspension in water. This difference must be attributed to the formation of radicals in water surrounding the cells. Direct hæmocytometer counting of the yeast cells showed that, under the wet condition of irradiation used here, the number of cells per ml. was about 5×10^7, that is, on the average the volume of water available per cell was 2×10^4 μ^3.

From the results with dry yeast, the sensitive volume per yeast cell could be estimated from Lea's theory[1]. This volume was found to be $2{\cdot}84 \times 10^{-4}$ μ^3 with a corresponding radius of 40·7 mμ. Thus the available volume of water was about 10^8 times the target volume.

The number of radicals formed per μ^3 with the given dose of irradiation could be estimated[6], and knowing the number of cells per unit volume, one can estimate the probability of inactivation per radical. This was found to be $3{\cdot}85 \times 10^{-9}$.

I am indebted to Prof. N. N. Das Gupta for directing the investigations, also to Dr. A. K. Sarma of the Botany Department and to Mr. S. Bhattacharjee for valuable discussion.

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[1] Lea, D. E., "Action of Radiation on Living Cells" (Camb. Univ. Press, 1955).
[2] Hutchinson, F., Preston, A., and Vogel, B., *Rad. Res.*, **7**, 465 (1957).
[3] Gunter, E. S., and Kohn, H. I., *Bacteriol.*, **71**, 571 (1956).
[4] Elkind, M. M., and Beam, C. A., *Rad. Res.*, **3**, 88 (1955).
[5] Uretz, R. B., *Rad. Res.*, **2**, 240 (1955).
[6] Read, J., *Phys. Med. Biol.*, **1**, 209 (1957).

Interstrain Ovarian Transplantation in Lethally Irradiated Mice After Bone Marrow Therapy

Since Lorenz *et al.*[1] demonstrated that survival time of lethally irradiated mice is increased by intravenous injection of bone marrow, it has been shown that this phenomenon results from repopulation of hæmatopoietic tissues by the injected cells (see Congdon[2] for review). Main and Prehn[3] reported that irradiated mice of the *DBA/2JN* strain that had been given bone marrow from (*BALB/cAnN* × *DBA/2JN*)F_1 hybrids accept grafts of *BALB/cAnN* skin. Such grafts usually fail both in irradiated animals and in irradiated animals that have received injections of isologous bone marrow. Trentin[4] showed further that irradiated strain *CBA* mice that had been given bone marrow from strain *C3H* donors accept skin grafts from both *CBA* and *C3H* animals. Similarly, Barnes *et al.*[5] found that irradiated *CBA* animals given strain *A* bone marrow succumb when inoculated with tumours specific for either of these strains. The experiments described here show that lethally irradiated (101 × *C3H*)F_1 female mice given strain *A* bone marrow will accept ovarian transplants from either (101 × *C3H*)F_1 hybrids or strain *A* donors. These treated females are capable of giving birth and of nursing and raising young.

Hybrid females, (101 × *C3H*)F_1 about 12 weeks old were exposed to 950 r. whole-body X-irradiation. Strain *A* bone marrow was injected (1 or 10 femur equivalents, that is, 10^7 or 10^8 cells, respectively) into the tail vein within 4 hours after exposure. These animals later received transplants of ovarian tissue from either strain *A* or (101 × *C3H*)F_1 donors. Three groups of control animals were used, all of which received 75 r. of X-rays (whole body) but no marrow therapy. These animals were given one of three treatments: (1) no ovarian transplant; (2) transplants from (101 × *C3H*)F_1 donors; or (3) transplants from strain *A* donors. In all operations half an ovary was transplanted into the ovarian capsule. The host ovary was not removed. Operations were unilateral in all cases. Nembutal was used for anæsthesia The results of our experiment are shown in Table 1.

The control experiments incorporate a modification of the ovarian transplantation technique developed by Parrot and Parkes[6,7]. Complete removal of ovarian tissue is difficult. It has been possible only by use of markers maintained by forced heterozygosis[8] to distinguish unequivocally between young produced from the transplanted ovary and those from the host ovary. All available evidence indicates that sterility induced by radiation in the female mouse is permanent even after low doses of radiation. If males are provided immediately after treatment, one or more litters, depending on the dose of radiation, may be produced prior to loss of fertility. If the addition of males is delayed, females lose fertility without producing young. Females so sterilized can be used in isologous ovarian transplanation experiments, and, if the interval between irradiation and mating is sufficiently long, the only functional ova produced will have arisen from transplanted ovarian tissue. Since host ovaries need not be removed and surgery may be limited to one side, trauma to the animal is minimized.

Females sterilized by radiation are capable of producing young from transplanted isologous ovaries. This, at a much lower dose level, is in accord with the results of Parrott and Parkes[6] in *R* mice after 900 r. In our series 3 of the 6 females given isologous ovarian transplants and mated 42 days after 75 r. each produced 7 litters (26, 34, and 31 young); 3 of the 4 mated 146 days after irradiation each produced 3 litters (12, 12, and 6 young). It has been observed by L. B. Russell and K. F. Stelzner (in the press), however, that 9 per cent of female mice exposed to 100 r. were still producing litters from matings made 45–65 days after irradiation. The proof, therefore, of the isologous takes in controls rests primarily on the 4 animals mated at 146 days after irradiation. As would be expected from principles of tissue transplantation, no young were obtained in the homologous transplants (strain *A* ovary into (101 × *C3H*)F_1 females irradiated with 75 r.).

Results from experiments in which strain *A* bone marrow therapy was given after 950 r. of X-rays are listed in Table 1. These females were all mated to albino males. Since strain *A* is homozygous for the gene for albinism and (101 × *C3H*)F_1 hybrids carry the allele for full colour at this locus, young from transplanted strain *A* ovary will be albino and those from (101 × *C3H*)F_1 ovary will be full-coloured. All young

Table 1. Transplantation of Homologous Ovary

No. of (101 × C3H)F_1 mice	Dose of strain A bone marrow (femur equivalents)	Time interval (days): Irradiation to ovarian transplant	Time interval (days): Irradiation to mating	Type of ovary transplanted	No. of females that had young
		Dose of X-rays : 75 r. (250 kVp, 30 mamp., 95 r./min.)			
10 { 6	None	—	42	None	0
10 { 4	None	—	140	None	0
10 { 6	None	13	42	(101 × C3H)F_1	3
10 { 4	None	15	146	(101 × C3H)F_1	3
10	None	132	139	A	0
		Dose of X-rays: 950 r. (250 kVp, 30 mamp., 90 r./min.)			
5	10	0	32	A	2
14 { 10	1	45	62	A	5
14 { 2	1	64	107	A	1
14 { 2	1	108	114	A	0
10	1	109	114	(101 × C3H)F_1	4

from females into which strain *A* ovary was transplanted (12 litters, 28 young) were albino. Young from females into which (101 × *C3H*)F_1 ovary was transplanted (8 litters, 16 young) were all full-coloured. These irradiated females thus accepted both isologous ovarian tissue and ovarian tissue from the strain that served as the source of bone marrow. Whether these animals will also accept ovarian tissue from other strains has not been determined.

Krohn[9] reported successful inter-strain transplantation of ovarian tissue from females of strains *C3H* and *CBA* to strain *A* females, which had been made 'tolerant' by spleen injection. Two means for making homologous transplantation of ovarian tissue possible are therefore now available – induced tolerance and lethal irradiation followed by bone marrow therapy.

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[1] Lorenz, E., Uphoff, D., Reid, T. R., and Shelton, E., *J. Nat. Cancer Inst.*, **12**, 197 (1951).
[2] Congdon, C. C., *Blood*, **12**, 746 (1957).
[3] Main, J. M., and Prehn, R. T., *J. Nat. Cancer Inst.*, **15**, 1023 (1955).
[4] Trentin, J. J., *Proc. Soc. Exp. Biol. Med.*, **92**, 688 (1956).
[5] Barnes, D. W. H., Ford, C. E., Ilbery, P. L. T., Koller, P. C., and Loutit, J. F., *J. Cell. Comp. Physiol.*, **50**, Suppl. 1, 123 (1957).
[6] Parrott, D. M. V., and Parkes, A. S., *Brit. Vet. J.*, **112**, 550 (1956).
[7] Parrott, D. M. V., and Parkes, A. S., *J. Endocrin.*, **14**, xxxvi–xxxvii (1957).
[8] Russell, W. L., and Hurst, J. G., *Proc. U.S. Nat. Acad. Sci.*, **31**, 267 (1945).
[9] Krohn, P. L., *Nature*, **181**, 1671 (1958).

Determination of Chlorine in Muscle Tissues by Neutron Activation Analysis

Neutron activation analysis permits the determination of sodium, potassium and phosphorus in exceedingly small samples of tissues, for example, needle biopsy material from human skeletal muscle[1]. Such measurements may be of value for the investigation of the electrolyte content of the cells. However, determination of the chloride content of the tissues would also be of great interest. The chlorides are predominantly present in the extracellular fluid and, thus, by determination of the chlorides in whole tissues and in plasma the distribution of electrolytes and water between cells and extracellular fluid can be calculated[2].

When chlorine is bombarded with neutrons in a reactor some of it is transformed into the isotope chlorine-38. This isotope has a half life of 37·3 min., and emits gamma quanta of 1·60 and 2·15 MeV. By gamma spectrometry of a muscle sample and of simultaneously irradiated standards of sodium and chloride the chlorine content of the sample can be determined.

The following procedure was used in our laboratory. Dried and fat-extracted muscle samples (1–2 mgm.) were weighed into small polyethylene containers. Other polyethylene containers with known amounts of sodium phosphate and potassium chloride, respectively, were placed near the sample containers into a large container suitable for introduction into the reactor. The irradiations took place in the reactor *R* 1 of AB Atomenergi, Stockholm. The containers were bombarded with neutrons at a flux of 5–6 × 10^{11} n/cm.^{2}/sec. for 40 min. After conclusion of the irradiation they were brought to the laboratory as quickly as possible, usually within 15 min. The samples were rapidly dissolved in 1 *N* sodium hydroxide solution on a boiling water bath. The standards were dissolved and diluted in water.

The measurements were made in a well-scintillation detector with a single channel pulse-height analyser. To avoid interfering, high-energy beta rays from potassium-42 the solutions were placed in a 2·75 mm. thick aluminium tube inside the well during the measurement. With this precaution practically all the counts above an energy level of 1·8 MeV. were due to radiation from chlorine-38 and sodium-24.

The first measurement of the sample and standards were begun as soon as possible, usually 30–40 min. after the neutron bombardment. For this counting a 'window' between 1·80 and 2·30 MeV. in the gamma spectrum was selected. After 8 hours, when the activity from chlorine-38 had disappeared, the samples and standards were again counted either in a 'window' including all the gamma radiation above 1·8 MeV. or in a 'window' selected to include only the 2·76 MeV. photopeak of sodium-24.

From the second measurement it was possible to calculate the ratio between sodium-24 in the sample and in the standard. As this ratio was the same in the first measurement it then could be calculated how

much of the first count was due to sodium-24. The count from chlorine-38 then could be obtained by subtraction. By comparison with the standard chloride count the chloride content of the sample was obtained.

Analysis of known amounts of chloride in standards made up of sodium phosphate and potassium chloride in mixtures gave a linear relationship between the amounts of chloride and the c.p.m. from chlorine-38.

Numerous determinations on a muscle preparation gave values which did not differ more than ±4 per cent from the chloride content determined by use of chemical methods. Preliminary results on human muscle biopsies gave values in accordance with results published by investigators using chemical methods.

A more detailed description of the method will be published later. I acknowledge the valuable help of the engineering staff of the reactor *R* 1 of the AB Atomenergi, Stockholm.

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[1] Reiffel, L., and Stone, C. A., *J. Lab. Clin. Med.*, **49**, 286 (1957).
[2] Mancry, J. F., *Physiol. Rev.*, 34, 334 (1954).

Cellular Uptake of Deoxyribonucleic Acid by Human Tissue Culture Cells

THE demonstration of the role of deoxyribonucleic acid in the transfer of genetic information has been one of the most important developments in modern biology. In bacteria the physical transfer of deoxyribonucleic acid may take place by non-cellular means[1-3]. In higher organisms, the transfer of genetic information, at least in the sexual cycle, requires a special cell, the gamete, produced in meiosis. However, the possibility of non-cellular transfer of genetic information in higher organisms, at least in somatic tissue, must be considered. Two attempts to transfer genetic information in higher organisms by injections of deoxyribonucleic acid into intact animals have already been reported[4]. The results of these experiments were conflicting and it seems that further work at a cellular level might be more fruitful. Mammalian tissue culture in conjunction with the use of labelled homologous deoxyribonucleic acid offers a system in which the possibilities of extracellular transfer of deoxyribonucleic acid in higher forms can be investigated. In this communication the results of such experiments utilizing the Henle tissue culture cell line (derived from human embryonic jejunum) and homologous deoxyribonucleic acid labelled with tritiated thymidine are reported.

The Henle cell line was obtained from Microbiological Associates and was grown at 37° C. in medium 199 (Microbiological Associates) plus 15 per cent inactivated human serum with 125 mgm./l. of streptomycin and pennicillin and 10 mgm./l. of chloromycetin. Cells were kept in one-litre Roux bottles containing approximately 90 ml. of complete medium. The deoxyribonucleic acid of the cells was labelled by adding tritiated thymidine (0·360 mc./m. mole, Schwarz Biochemicals) to the medium to make a final concentration of approximately 0·05 μc./ml. The harvested cells were washed repeatedly with saline, lysed with the aid of an anionic detergent, centrifuged and the crude deoxyribonucleic acid precipitated from the supernatant by the addition of an equal volume of 95 per cent ethanol. Although this preparation contained considerable amounts of protein and ribonucleic acid, the thymidine label was only in the deoxyribonucleic acid and consequently further purification was not necessary. All samples were counted in a liquid scintillation counter (Packard Instrument Co., Inc.) using a dioxane system as solvent and diphenyloxazole as phosphor[5].

The labelled deoxyribonucleic acid preparation was injected into the medium of a growing culture (approximately 2×10^7 cells) to make a final concentration of approximately 0·2 μgm./ml. After one hour incubation at 37° C. in the presence of labelled deoxyribonucleic acid, the cells were harvested, washed repeatedly with saline (5 times), and/or incubated with deoxyribonuclease (0·1 mgm./ml.). The washed cells were then lysed by repeated freeze-thawing cycles (10 times) and the amount of label taken up by the cells determined on the resulting suspensions. In three separate experiments, the percentage of label taken up by the cells under these conditions was 0·3, 1·2 and 0·8 per cent respectively. If after exposure to labelled deoxyribonucleic acid, the medium is replaced with fresh unlabelled medium and the cells permitted to continue growing, it can be shown that at least a part of the label taken up by the cells is incorporated into the host's deoxyribonucleic acid. In one experiment where approximately 1·0 ml. of packed cells were grown under these conditions, deoxyribonucleic acid extracted from the cells was labelled to the extent of 0·14 per cent of the original label introduced into the medium. Thus it is clear that label from extracellular deoxyribonucleic acid can be taken up and incorporated into cellular deoxyribonucleic acid by these cells.

However, this specifies nothing about the form in which the label is taken up by the cells. Clearly, the important point is whether the incorporated deoxyribonucleic acid label represents degradation products or relatively intact molecules of the extracellularly administered deoxyribonucleic acid. Two lines of evidence bear on this point: (1) the relative stability of extracellular labelled deoxyribonucleic acid under the experimental conditions; and (2) the effect of unlabelled deoxyribonucleic acid, thymidylic acid, thymidine, and thymine on the cellular incorporation of labelled deoxyribonucleic acid.

(1) Undegraded labelled deoxyribonucleic acid in the presence of protein included in the medium is completely precipitable by 95 per cent ethanol. One hour following injection of labelled deoxyribonucleic acid, label in the medium is still completely precipitable with 95 per cent ethanol which suggests that uptake of label during this period may involve undegraded deoxyribonucleic acid.

(2) More important for this point are the experiments involving the effect of cold thymine, thymidine, thymidylic acid, and deoxyribonucleic acid on the incorporation of labelled deoxyribonucleic acid, the results of which are given in Table 1. As can be seen, thymine, thymidine, and thymidylic acid have no effect on the uptake of label, whereas unlabelled deoxyribonucleic acid effectively reduces such uptake.

Table 1. EFFECT OF AN EXCESS OF UNLABELLED THYMINE, THYMIDINE, THYMIDYLATE AND DEOXYRIBONUCLEIC ACID ON THE UPTAKE OF LABELLED DEOXYRIBONUCLEIC ACID (DNA)

Counts per minute taken up by cells in the presence of:				
Labelled DNA alone	Labelled DNA + 100-fold excess of thymine	Labelled DNA + 100-fold excess of thymidine	Labelled DNA + 100-fold excess of thymidylate	Labelled DNA + 100-fold excess unlabelled DNA (calf thymus)
705	700	720	710	142

These results are what one would expect if the uptake of label involved undegraded deoxyribonucleic acid and not its degradation products.

These experiments indicate that uptake of extracellular undegraded deoxyribonucleic acid by mammalian cells in tissue culture is possible. Whether the demonstrated incorporation of label by the cell's deoxyribonucleic acid also involved undegraded extracellular deoxyribonucleic acid is not clear. Experiments are now being considered to examine this important point, and to find the optimal conditions for mammalian cellular uptake of undegraded deoxyribonucleic acid.

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[1] Avery, O. T., MacLeod, C. M., and McCarty, M., *J. Exp. Med.*, **79**, 137 (1944).
[2] Catlin, B. W., and Cunningham, L. S., *J. Gen. Microbiol.*, **19**, 522 (1958).
[3] Zinder, N. D., and Lederberg, J., *J. Bacteriol.*, **64**, 679 (1952).
[4] Benoit, J., Leroy, P., Vendrely, C., and Vendrely, M. R., *C. R. Acad. Sci. Paris*, **247**, 1049 (1958). Medawar, P. B., *Nature*, **182**, 62 (1958).
[5] Davidson, D., and Feigelson, P., *Int. J. App. Rad. Isotopes*, **2**, 18 (1957).

Radiomimetic Action of Lipoxidase

In the course of our researches on the role of lipoperoxides in animal organisms, we have worked with lipoxidase. The lipoxidase used was obtained from soya bean by Theorell, Homan and Akeson's technique[1], slightly modified. The animals employed were white mice weighing 20–30 gm. and rats weighing 100–200 gm.

Large intraperitoneal doses of lipoxidase (300–500 mgm./kgm.) induced in the test animals a period of excitation soon followed by prolonged somnolence. Even larger doses of lipoxidase given by the same route sometimes caused paresis of the hind legs. Diarrhœa was present in all cases. After a few hours a progressive dyspnea appeared, which in some animals terminated with death. The outstanding finding at post-mortem was a large serous accumulation in the pleural cavity.

The chronic toxicity of lipoxidase was studied by adding it to the drinking water of the test animals (1 mgm./ml.). Five white mice were given this water for a period of 30 days. The interesting fact, however, was that 21 days after the termination of the administration of lipoxidase in drinking water, all the animals showed a profuse loss of hair affecting mainly the sides, back and head. Histological examination of the alopecic patches shows a very marked atrophy of the hair follicles and probably a secondary-thinning of the corion (Fig. 1).

There is abundant reference in the literature to the relationship between the effects of Röntgen radiation and the formation of peroxides. Gerschman *et al.*[2] pointed out the possibility of a common mechanism in the action of radiation and in oxygen intoxication. There are several points of similarity between lipoxidase intoxication and the effects of X-rays. For example, alopecia, somnolence, paresis and diarrhœa. Rats submitted to high oxygen pressures show pulmonary changes similar, if milder, than those described above[3]. This has been called the Lorrain-Smith effect[4]. Moreover, a so-called 'radiation pneumonitis' has been reported in the course of general and of thoracic radiation therapy[5], and the synergism between hyperoxygenation and radiation is well known[6].

All these facts may be connected with the phenomena resulting from administration of lipoxidase, their common characteristic being the formation of peroxides. The likelihood of lung involvement is understandable since it is in this organ that direct contact with atmospheric oxygen takes place and oxygen exchange is more active than elsewhere in the organism. Accordingly, we decided to investigate the suspected connexion between the effects of lipoxidase and those of X-rays. Three groups of 10 rats each were employed. All animals were from the same strain, Group 1 animals received a sublethal dose (250 mgm./kgm.) of lipoxidase. Group 2 were irradiated (495 r.; 200 kV.; filter 1 mm. copper plus 2 mm. aluminium; focal distance 85 cm.; intensity 5·5 r./minute), and group 3 rats received both the lipoxidase dose and the irradiation. After two months, all the animals in group 1 survived, one rat in group 2 died on the fourteenth day after treatment, while

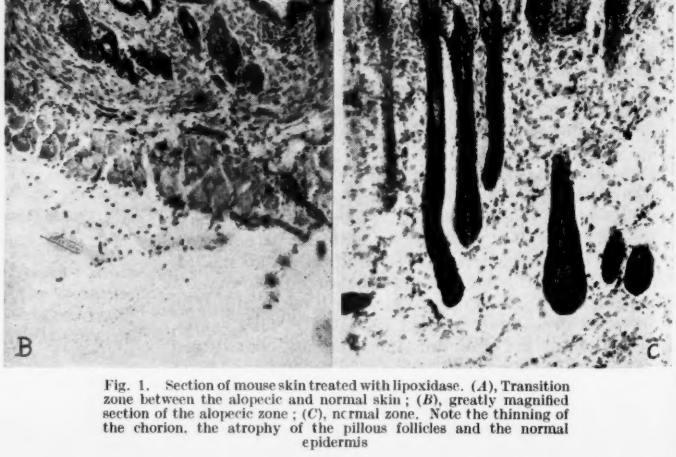

Fig. 1. Section of mouse skin treated with lipoxidase. (*A*), Transition zone between the alopecic and normal skin; (*B*), greatly magnified section of the alopecic zone; (*C*), normal zone. Note the thinning of the chorion, the atrophy of the pillous follicles and the normal epidermis

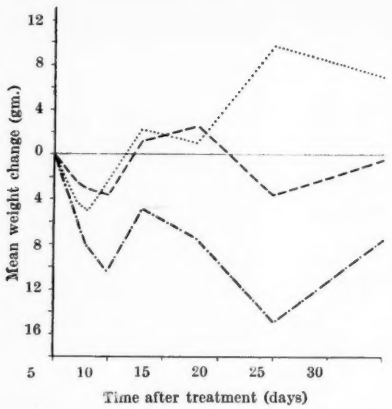

Fig. 2. Change in weight of rats treated with: ....., X-rays (495 r.); — — — lipoxidase (250 mgm./kgm.); · — · — · — · — · —, X-rays and lipoxidase. Note the greater loss of weight in the last group, and the slow recovery of these animals

6 animals in group 3 died within 48 hours and, although the rest survived, they clearly showed the ill effects of the treatment (Fig. 2). It can be argued that even if the actions of lipoxidase and of radiation were different their effects might still reinforce each other. We do feel, however, that there are reasons to think otherwise.

In vitro, we obtained similar quantities of peroxide oxygen (estimated with linoleic acid) by the action of 38 μgm. lipoxidase for 9 minutes, by 3,100 r. X-rays, and by the passage of 243 l. of oxygen.

We are indebted to Dr. J. Rubio for the histological preparations and to Dr. M. Badell for the X-ray treatments. We also want to thank Prof. F. G. Valdecasas for his comments and keen interest on our work.

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[1] Theorell, H., Holman, R. T., and Akerson, A., *Acta Chem. Scand.*, **1**, 574 (1947).
[2] Gerschman, R., Gilbert, D. L., Nye, S. W., Dwyer, P., and Fenn, N. O., *Science*, **119**, 623 (1954); *Proc. Soc. Exp. Biol. Med.*, **86**, 27 (1954).
[3] Puig Muset, P., and Valdecasas, F. G., *Trabajos Inst. Nac. Ciencias Med.*, **4**, 389 (1946).
[4] Bean, J. W., *Physiol. Rev.*, **25**, 1 (1945).
[5] Whitfield, A. G. W., Bond, W. H., and Arnott, W. M., *J. Rad.*, **6**, 12 (1954). Brown, R. F., *Amer. J. Roentg.*, **75**, 976 (1956).
[6] Churchill-Davidson, I., Sanger, C., and Thomlinson, R. H., *Lancet*, i, 1091 (1955); ii, 1107 (1958).

BIOLOGY

Dissociation of the 'Surface Membrane Complex' in Atrophic Muscle Fibres

When one examines the cross-section of a muscle fibre with the electron microscope, a 'membrane-complex'[1] of the type shown in Fig. 1 is observed, consisting of two dense layers separated by a light zone of about 200–300A. width. The sections shown in Figs. 1–3 has been fixed in osmium tetraoxide and stained with phosphotungstic acid in alcohol. (All scales equal 1μ.) There are reasons for regarding only the inner layer as a 'physiological' cell membrane and the outer layer as an extra-cellular component, frequently described as a 'basement membrane'[2]. Some authors even regard the outer layer as a fixation artefact. The two layers can be distinguished, not only by their different appearance (the outer layer is more 'fuzzy', tending to fray out and connect with fine connective tissue fibrils), but also by their different 'electron-staining' properties. Thus, in permanganate preparations in which cellular and mitochondrial membranes show up in good contrast, the outer layer is usually barely visible, and in general, its relative density under different conditions of fixation and staining corresponds more closely to that of the protein filaments in the muscle fibre than to the cellular or intra-cellular membrane structures in muscle, nerve or Schwann cells (Birks, Huxley & Katz, unpublished work).

The purpose of this communication is to describe a striking change in the relation between the two surface structures observed during an advanced state of muscle atrophy. Denervated sartorius muscles of frogs which have been kept at 20°C. for more than four months show a variable degree of atrophy; some muscles become very thin, and their fibres much reduced in size. Changes in the muscle fibre surface are at first confined to longitudinal 'wrinkling', that is, formation of shallow folds which run parallel to the fibre axis, without altering the mutual relation of the two surface layers. A more advanced stage is illustrated in Figs. 2 and 3; in this muscle many fibres had

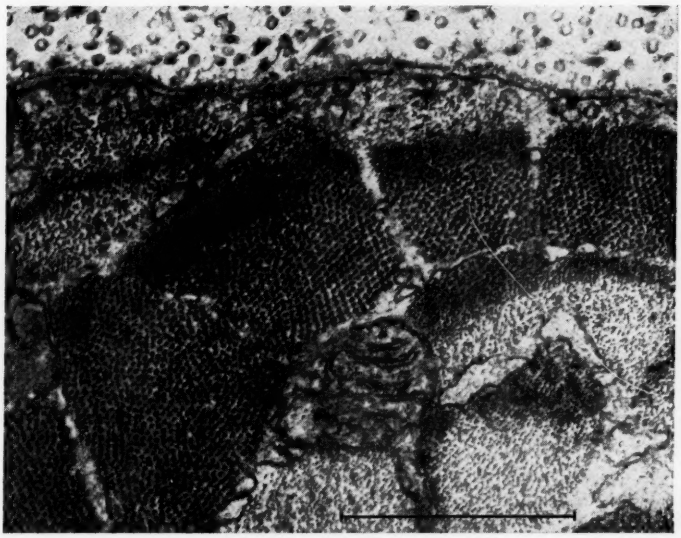

Fig. 1. Portion of a normal muscle fibre of the frog with its 'surface membrane complex

Fig. 2. Atrophied muscle fibre. Outer surface layer is partly detached and folded. The double-array of myofilaments is still visible in patches

shrunk to a diameter of several microns (approximately a tenth of their normal size), and the characteristic fibrillar structures were either absent or only visible in patches.

In such highly atrophic muscle fibres, the cell membranes (inner layer) are found to have shrunk down to the reduced diameter and show little wrinkling; but the basement membranes of the individual cells (outer layer) appear to have undergone relatively little shrinkage. They have become partially detached from the cell surface and form an elaborate system of longitudinal pleats which, if unfolded, would fit a fibre of more nearly normal size.

These observations suggest that the external layer, far from being an 'artefact', is indeed a stable structure, and that it is quite separate from the cell membrane which it closely invests under normal conditions. As regards the functional significance of this layer, little can be said; but its presence in the synaptic space between nerve and muscle (ref. 3; Birks *et al.*, unpublished work) makes it very difficult to believe that it can act as a diffusion barrier to molecules of the size of acetylcholine or even curarine.

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[1] Robertson, J. D., *J. Biophys. Biochem. Cytol.*, **2**, 369 (1956).

[2] Robertson, J. D., 'Biochem. Soc. Sympos.', **16**, 3 (1959).

[3] Robertson, J. D., *J. Biophys. Biochem. Cytol.*, **2**, 381 (1956).

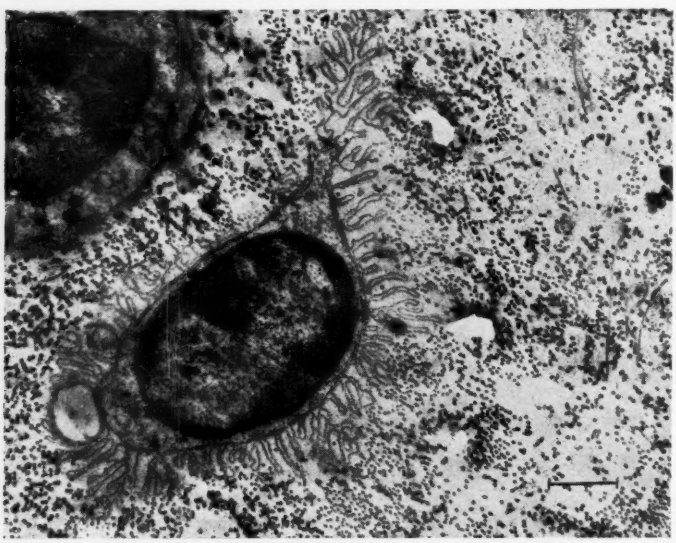

Fig. 3. Survey picture, at low magnification, of a highly atrophic fibre. The fibre is surrounded by masses of collagen. Its interior is occupied by a nucleus; myofilaments cannot be recognized. The outer surface layer forms a complicated system of 'pleats'

Use of Transparent Cellulose Film for Roller Tube Tissue Cultures

For many years a plasma clot has been used as the solid medium to support the growth of cells *in vitro*, and has proved eminently suitable in many respects. It is well known, however, that plasma suffers from some disadvantages, the chief of which, until recently, have been the difficulty of its collection and the necessity for frequent patching of the clot to prevent detachment and loss of cultures.

With the application of new techniques of investigation, the presence of a plasma clot is tending to become even more troublesome; Crain[1] and Hild, *et al.*[2] have commented adversely on the effect of plasma when performing micromanipulation on tissue culture cells; the application of histochemical methods may be invalidated by the presence of the plasma; and, finally, the fact that the cells are embedded in a protein-containing medium is positively inimical to the use of microscopic interferometry or refractometry.

Rose, *et al.*[3] have recently advocated the use of strips of 'Cellophane', instead of plasma, as a means of supporting the growth and differentiation of cells in a perfusion chamber. The present communication is concerned with the extension of this idea to the flying coverslip roller tube method.

Strips are cut from 'Visking' dialysis tubing; they should be slightly smaller than the 12×50 mm. coverslips normally used in roller tubes. The strips are sterilized and prepared, following the procedure described in ref. 3. The explants are arranged on a 12×50 mm. coverslip, and a 'Cellophane' strip carefully lowered on top of them; the strip is then

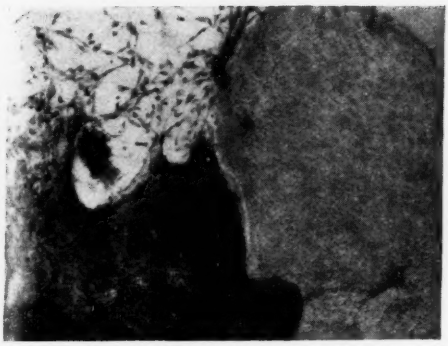

Fig. 1. Mixed growth of fibroblasts and epithelial cells in a 2-day old culture of kidney explanted from a 2-day old rat. Jacobson's stain

held in position against the coverslip by gripping the two at each end with a sterile tantalum clip of the type used in surgery for occluding blood vessels. The two clips are perfectly adequate to hold the 'Visking' strips securely in position, provided that they are closed firmly. The coverslip is then placed in a sterile test tube with 2 ml. of fluid medium, and inserted in the roller drum at 37°C.

When the cultures have achieved a suitable state of growth, the tantalum clips are slipped off (and may be used again), and the strips gently lifted away. The explants in most cases remain attached to the glass coverslip; the cells in the out-growth invariably do so. The cultures may then be rinsed in isotonic saline, and either transferred to a perfusion chamber for examination in the living state by phase contrast or interference microscopy, or subjected to routine fixation and staining or to histochemical procedures.

Of the various tissues which have so far been cultured by this method, moderate to profuse growth has been produced in all but fœtal or young adult brain, which, because of its extreme softness, tends to become flattened by the 'Visking' strips. Fig. 1 illustrates the growth obtained by the method.

It is hoped that this extension of the original idea of Rose and his colleagues to the roller tube will enable it to be more widely used, particularly when cultures are desired which are capable of giving the maximum information via histochemical, interferometric or micromanipulative techniques. The method has the added attractions of (*a*) making the collection and storage of plasma unnecessary, (*b*) obviating the need for patching of cultures, and (*c*) removing an uncontrolled variable from the environment of the cells.

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[1] Crain, S. M., *J. comp. Neurol.*, **104**, 285 (1956).
[2] Hild, W., *et al.*, *Experentia*, **14**, 220 (1958).
[3] Rose, G. G., *et al.*, *J. Biophys. Biochem. Cytol.*, **4**, 761 (1958).

Feeding Mechanism of *Tilapia nigra*

Examination of the gut and rectal contents of *Tilapia nigra* (Günther) has shown that this fish, like *T. esculenta* Graham[1], feeds mainly on phytoplankton or epiphytic algæ, digesting the diatom fraction, but passing the green and blue-green algæ untouched through the gut. (Rectal smears from *T. nigra* have in fact yielded cultures of both green and blue-green algæ, as has been reported for the closely related *T. mossambica* Peters[2].) *T. esculenta*, which has simple and moderately widely spaced gillrakers, feeds on suspended plankton[3], and this fact has led to speculation on the method by which the finer food particles are strained out. Greenwood[4] considered the interdigitation of the gillrakers sufficient to retain only the coarser elements which sometimes appear in the stomach, for example, copepods. He suggested that the smaller particles are entangled in a film of mucus secreted especially by cells lying along the posterior mid-dorsal surface of the mouth and pharynx and on the gill arches.

Gosse[5], working on *T. nilotica* (L.) and *T. melanopleura* A. Dum., discovered micro-branchiospines on the outside faces of all but the first gill arch, and he suggested that the filtration of small food particles (down to 10μ in diameter) was achieved by means of these minute structures.

Examination of the gill arches of *T. nigra* has shown that essentially similar micro-gillrakers are present. In fishes more than 200 mm. in standard length, a series of about a hundred micro-gillrakers lie at the bases of the gill filaments on the external faces of the 2nd, 3rd and 4th gill arch, each micro-raker being directed forward and bearing a series of 8–10 teeth on either side (Fig. 1). The teeth are 25μ in length, tapering to points and curved forwards, and in most cases they meet or even overlap the adjacent series. In a fish of 240 mm. standard length, the micro-rakers were 250μ in length and 100μ in width. In specimens less than 100 mm. in standard length the teeth are reduced in size and number, only 4–5 being present in fishes of 40 mm.

Gosse suggested that the micro-gillrakers function as passive sieves, the water being forced to pass between each raker during its passage toward the gill filaments. However, the shape and position of the rakers suggest equally that they may actually function as rakers, actively combing the inner face of the next gill. In this way they would serve both to concentrate the finer food particles entangled in the mucus, and also

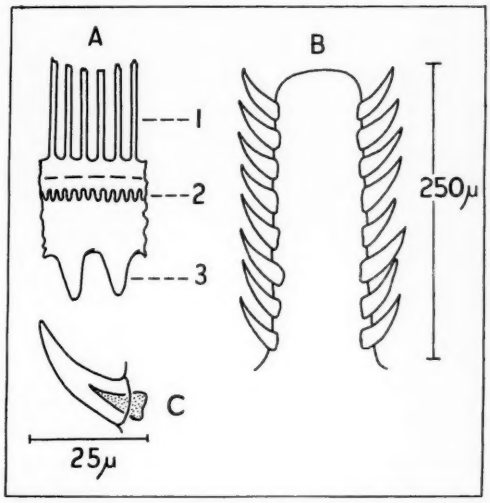

Fig. 1. The micro-gillrakers of *Tilapia nigra*. *A*, portion of second gill arch (exterior face); *B*, single micro-gillraker from specimen 240 mm. standard length; *C*, single tooth. (1) basal part of gill filaments; (2) band of micro-gillrakers; (3) gillrakers

to push the mucous film forward against its tendency to escape with the exhaled stream of water. Small particles and unicellular algæ have been found associated with the micro-gillrakers, although not in the quantities one might expect. The final collection and concentration of food is probably achieved by the pharyngeal apparatus[4].

I have found micro-gillrakers in *T. nigra*. *T. mossambica*, *T. esculenta*, *T. variabilis* Blgr., *T. zillii* Gervais, and *T. melanopleura*. The shape of the micro-rakers and the teeth is identical in all these species, but in *T. variabilis*, *T. zillii* and *T. melanopleura* there are usually more teeth (10–16; Goose records 18 in *T. melanopleura* and *T. nilotica*). Adult *T. zillii* and *T. melanopleura* feed mainly on higher plants, but phytoplankton has been recorded as a subsidiary food in the latter[3]. Micro-rakers were not found in *T. grahami*, Blgr., a small species confined to the strongly alkaline waters of Lake Magadi, Kenya. They are however present in at least one species of *Haplochromis*, *H. bloyeti* (Sauvage)—again identical to those of *T. nigra*. In the cyprinid *Labeo cylindricus* Peters, an algal grazer, micro-rakers are absent. In this fish the gillrakers are very close set (about 60 on the lower part of the anterior arch) and probably provide a sufficiently fine filtration surface.

Micro-gillrakers may hold some systematic value (*vide* the greater numbers of teeth in certain species). They may however be of more importance at generic levels, particularly where specialization has been towards a piscivorous or molluscivorous habit. A rather simpler type of micro-raker is found in species of *Citharinus* (Characidæ), another filter feeder[5].

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[1] Fish, G. R., *Nature*, **167**, 900 (1951).
[2] Vaas, K. F., and Hofstede, A. E., *Contr. Int. Fish Res. Sta. Bogor*, **1**, 1 (1952).
[3] Fish, G. R., *Uganda J.*, **19** (1), 85 (1955).
[4] Greenwood, P. H., *Nature*, **172**, 207 (1953).
[5] Gosse, J.-P., *Soc. Roy. Zool. Belge*, **86**, 303 (1955).

Infection of Cattle with *Cysticercus bovis* by the Injection of Onchospheres

In 1907 Dévé[1] reported the successful infection of a rabbit by the subcutaneous injection of *Echinococcus granulosus* eggs which produced a single fertile hydatid cyst. Leonard and Leonard[2], studying the resistance of rabbits to the larvæ of *Tænia pisiformis*, infected these animals by the injection of hatched eggs into the mesenteric vein. Fedeli Avanzi[3] reported that the intraperitoneal injection of hydatid sand into rabbits failed to produce any infection, but Batham[4] successfully infected mice by the intraperitoneal injection of *E. granulosus* eggs.

In the annual report of the Kenya Veterinary Department for 1944[5] Mann is reported as having hatched *T. saginata* eggs in acid pepsin and pancreatin solutions, but the onchospheres failed to infect calves when injected intravenously or subcutaneously.

It has been found by Froyd[6] that adult cattle in Kenya are apparently resistant to infection with *T. saginata* eggs when these are given *per os*. A similar experience has been reported by Urquhart[7]. In order to discover whether adults were totally resistant to infection, parenteral routes were tried, since work on other parasites had shown that there is probably more than one phase of resistance to parasitic infection[2, 8, 9].

Table 1. Results of injection of hatched *T. saginata* onchospheres in adult cattle

Route of Infection	Artificial Infection		Result doubtful
	Successful	Unsuccessful	
Naturally preinfected cattle			
Intravenous	0	2	—
Subcutaneous	1	2	—
Subcutaneous and intramuscular	3	2	—
Cattle not preinfected			
intravenous	0	0	2, of which 1 died 11 days after injection
Subcutaneous	1	1	—
Subcutaneous and intramuscular	5	5	—
Total	10	12	2

To date, 24 adult cattle have been injected intravenously, subcutaneously and/or intramuscularly with onchospheres of *T. saginata*, hatched by Silverman's technique[10]. Some of these beasts were found to be naturally preinfected by *C. bovis*.

The results of these injections are shown in Table 1. Thus in ten animals cysts were found at the injection sites. One other, doubtful, beast injected intravenously had two small cysts in the intramuscular fascia of a hindleg, but their histological appearance was not sufficiently conclusive to allow a definite diagnosis of *C. bovis* infection.

In two cases viable cysts were demonstrated at the injection site. In several others macroscopical evidence of cysts was noted, proved histologically to be indistinguishable from cestode tissues and in some cases showing scolices, thus leading to a definite diagnosis of successful parenteral infection.

Batches of eggs vary in the viability of the onchospheres which they produce. Seven of the negative cases above belonged to one batch which, though infective for calves, failed to infect any adult bovine. Excluding that group our series gave 58·8 per cent infection instead of 41·7 per cent all inclusive.

A fuller report on the above experiments will be published elsewhere.

This letter is published with the permission of the Director of Veterinary Services, Kenya.

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[1] Dévé, F., *C. R. Soc. Biol.*, **63**, 332 (1907).
[2] Leonard, A. B., and Leonard, A. E., *J. Parasit.*, **27**, 375 (1941).
[3] Fedeli Avanzi, C., *Ann. Fac. Med. vet. Pisa*, **5**, 152 (1952) ; abstract in *Helminth. Abstr.*, **21**, 235 (1952).
[4] Batham, E. J., *N.Z. Vet. J.*, **5**, 74 (1957).
[5] Kenya Vet. Dep. Ann. Rep. 1944, 15 (1946).
[6] Froyd, G. (unpublished results, 1958).
[7] Urquhart, G. M. (private communication, 1959).
[8] Chandler, A. C., *Amer. J. Hyg.*, **22**, 157 (1935).
[9] Sarles, M. P., and Taliaferro, W. H., *J. Infect. Dis.*, **59**, 207 (1935).
[10] Silverman, P. H., *Ann. Trop. Med. Parasit.*, **48**, 207 (1954).

Conjugation in *Spirostomum*

Any report of conjugation in *Spirostomum* is of special interest because of its extreme rarity. To our knowledge the last report was by Bishop[3] nearly thirty-six years ago in *S. ambiguum*. Earlier, Stein[1] and Balbiani[2] had observed the process in the same species. In no case was it noticed as a regular phenomenon (as in *Paramecium* and other ciliates) affecting considerable numbers of animals in a population. Nor were any of these authors able to present a full account of the conjugation process, since it was encountered by them very infrequently. As Bishop says, 'There

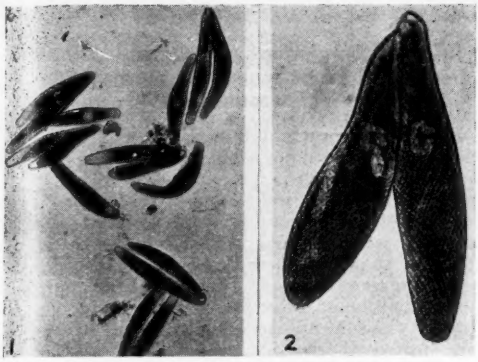

Fig. 1. Living conjugating pairs of *Spirostomum* sp. (× 8)
Fig. 2. A conjugating pair showing the macronucleus in each animal (× 32)

was no appearance of true epidemic' of conjugation.

The present communication reports the occurrence of conjugation in a new species of *Spirostomum* the cytology of which has been described earlier[4]. This appears to be the first instance in which the conjugating pairs were noticed in large enough numbers for the phenomenon to be regarded as a true epidemic.

Conjugation was never encountered in stock cultures obtained from a single natural source. Collections of *Spirostomum* from a number of different localities in India have been maintained in our laboratory under identical conditions. Mixtures from two stocks, Tirupati × Poona always yielded conjugants. Mixtures from other localities failed to produce conjugating pairs, although extensive tests were not made. If mating types occur in *Spirostomum*, Tirupati and Poona evidently represent two of them.

Conjugation was invariably preceded by a characteristic massing or agglutination of a large number of individuals, now regarded in *Paramecium* as the striking initial mating reaction. Several dozen animals stick together to form large masses or agglutinates. After 8–10 hours the clumps break down, releasing a large number of mating pairs. Two individuals of *Spirostomum* come together, spiral about each other for a few minutes and apply themselves along their peristomial margins. They move about in a well co-ordinated manner. At this stage the pairs are joined only by their cilia. After remaining together for a short time, they may separate and repeat the process with the same or with other individuals until finally union is effected involving adhesion of the bodies in the anterior region. Individuals of a pair severed before exchange of nuclear material live and divide in a normal manner.

It was noticed that the conjugants in a culture showed less variation in size than non-conjugants. Generally, the anterior end of one individual of the pair is attached a little behind the anterior end of the other. Evidently this is the holdfast region. The attachment ends at the cytostome, posterior to which the bodies of the conjugants are separate. The contractile vacuole seems to function in a normal manner during conjugation. The time taken from attachment of a pair of conjugants to their separation varies between 20 and 24 hr. Our observations on cultures maintained in the laboratory lead us to believe that conjugation occurs at fairly frequent intervals and a minimum period of 5 days elapses between successive conjugations. Every time conjugation occurred, it affected a large number of animals.

The species studied by Bishop[3] was non-viable following conjugation. In contrast, *Spirostomum* sp. used in our studies possesses a high degree of viability following conjugation.

Changes in the macro-nucleus are among the first internal processes during conjugation. It first condenses into a compact body much in the same manner as it does during binary fission. Later it undergoes fragmentation. In *Spirostomum* sp. all the micronuclei present in each conjugant appear to enter the first meiotic division, but not all these products divide again. The details of nuclear behaviour during conjugation are under investigation.

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[1] Stein, Fr., "Der organismus der Infusiontiere", **2** (Leipzig, 1868).
[2] Balbiani, F. G., *Ann. Micrographie.*, **4**, 369 (1892).
[3] Bishop, A., *Quart. J. Micro. Sci.*, **67**, 391 (1923).
[4] Seshachar, B. R , and Padmavathi, P. B., *J. Protozool.*, **3**, 145 (1956).

Resistance of *Sorghum* to Witchweed

Strains of *Sorghum vulgare* resistant to root parasite witchweed (*Striga lutea*) have been bred in South Africa over a number of years.[1] One of these (var. 'Framida') is characterized by its inability to germinate the parasite seed, which requires a stimulatory substance normally excreted from the host plant roots. A comparison of the resistant variety 'Framida', and a susceptible variety 'Light Mori' (from Nigeria) illustrates this point very clearly. Seedlings of the two varieties were grown in coarse washed sand in 3-in. plastic pots (50 seedlings per pot) for a period of nine days at ambient temperature (23–31°C.). After this the root solutions were eluted with 20 ml. of distilled water. The eluted solutions were tested for their ability to germinate the parasite seed in Petri-dish culture at full strength (as eluted) and at dilutions of 1:10 and 1:100. Typical results are shown in Fig. 1. It can be seen that the concentration of germination stimulent from the susceptible variety ('Light Mori') is supra-optimal and that a dilution of 1:10 does not reduce germination of the parasite seeds significantly. In the resistant variety, however, a similar dilution reduces germination almost completely ; while even at the highest concentration germination is significantly lower. Since it is unlikely that a concentration of root growth stimulant approaching that of the experimental conditions will be encountered in Nature, this seems to be at least one mechanism by which resistance is achieved.

The possibility of the lower germination by the resistant variety being due to the interaction of an inhibitor with the stimulatory substance was investigated. A comparison was made of the stimulating root solution from the susceptible variety diluted : (1) with water, and (2) with 'Framida' root solution. The results are shown in Fig. 2. The 'Framida' root solution causes some inhibition of germination, but this is not enough to account for all of the reduction shown in Fig. 1, which can only be explained by a lower production of the stimulatory substance. The 'Framida' solution was strongly colloidal, while that of the susceptible variety was less so. It is possible that inhibition is due to a proteinaceous compound similar to that from the hulls of *Avena sativa*, demonstrated by Elliot and Leopold[3].

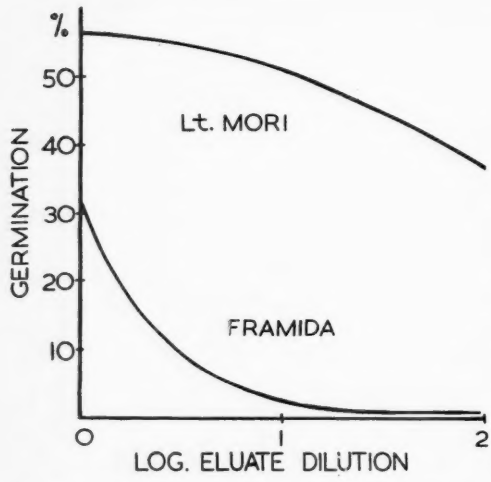

Fig. 1. Germination of *Striga lutea* seeds by root solution eluates of two varieties of *Sorghum vulgare* (var. Light Mori, a variety susceptible to *Striga* and var. 'Framida', a resistant variety).

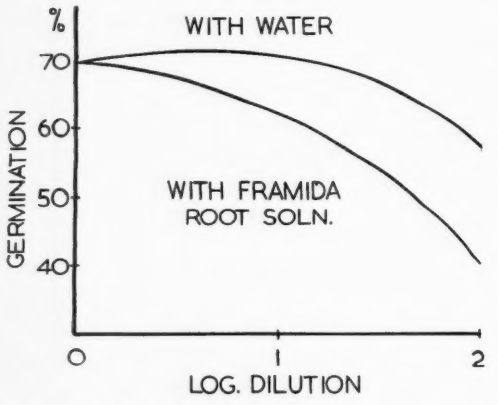

Fig. 2. Germination of *Striga lutea* seeds by root solution eluates of the susceptible variety ('Light Mori'); diluted with water (top curve) and with root solution from the resistant variety ('Framida') (lower curve).

This type of resistance is different from that of other sorghums bred in South Africa, in which mechanical and physiological obstruction is the cause of reduced *Striga* attack[1,2]. Resistance of the present type is likely to be successful against all species and physiological strains of *Striga*, and it is worth emphasizing the simplicity of testing a large number of varieties using the above technique.

I am grateful to Mr. D. Curtis of the Northern Nigerian Department of Agriculture, Research Headquarters, for supplying the seed.

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[1] Saunders, A. R., *South Afr. Dept. Agric. and For. Sci. Bull.*, **14**, (1942).
[2] Saunders, A. R., *Sci. Bull. Dept. Agric. South Afr.* (1933).
[3] Elliot, B. B., and Leopold, A. C., *Physiol. Plantarum*, **6**, 65 (1953).

SOIL SCIENCE

Effect of Emulsifiers and Organic Diluents in Soil Insecticide and Nematicide Formulations

Formulations of soil insecticides and nematicides should be used with discrimination as to soil condition and soil type.

Staniland and Stone[1] found that when equal volumes of (*a*) pure water (*b*) 1 per cent detergent solution were percolated through identical soil columns, 10 per cent less liquid passed through when using detergent solution. They stated that the higher surface tension of water causes it to run more rapidly through the soil capillaries and the soil particles were not wetted thoroughly, whilst the lower surface tension of the detergent solution causes it to be retained more in the soil. A lower surface tension however should mean a lower retention in the soil capillaries and should therefore lead to increased percolation rates.

Results in our laboratory, using a number of ethylene dibromide emulsions showed that the percolation rates of these emulsions through soil were much slower than that of pure water, the soil tending to retain the emulsions, and, as noted by Staniland and Stone using detergent solution, becoming spongy in texture. On using pure white sand columns however there was no great difference in percolation rates between ethylene dibromide emulsions and pure water. Thus, a change in soil structure appears to be the reason for slower percolation rates through soils when using emulsifiers.

On comparing different soil types in columns which had equal percolation rates with respect to water, it was found that ethylene dibromide emulsions passed through a sandy loam at a higher percolation rate than through a peaty soil in which the emulsion appeared to be retained more strongly and was broken to a greater degree in the soil. White sand columns did not break these emulsions. It may be noted that Fleming and Baker[2] using carbon disulphide emulsions in highly sorptive soils found that the maximum

Fig. 1. Sorption isotherms at 20° C. *A*, pure ethylene dibromide; *B*, ethylene dibromide + alcohols (*n*-butyl and *n*-hexyl); *C*, ethylene dibromide + hydrocarbons (petroleum ether and xylene)

penetration of lethal quantities against Japanese beetle larvæ was only 1–3 inches.

The U.S. Department of Agriculture[3] gives a formulation for use against Japanese beetle larvæ consisting of (percentages by weight): 2½ ethylene dibromide, 2½ detergent, 95 organic diluent (*iso*propyl alcohol). In our laboratory, formulations of 50 per cent ethylene dibromide (by weight) dissolved in organic diluents were allowed to vaporize in flasks containing a known weight of moist sandy loam soil and the sorption was determined by measuring the residual vapour concentration. The results were compared with that for pure ethylene dibromide. The sorption isotherms showed enhanced sorption of ethylene dibromide when it was dissolved in organic diluents, being 3 times greater with alcohols and 3½ times greater with hydrocarbons.

Thus, the use of emulsions and organic diluents may markedly affect the dispersion of the toxic agent through the soil.

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[1] Staniland and Stone, *J. Helminth*, **27**, Nos. 1 and 2, 41 (1953).
[2] Fleming and Baker, Tech. Bull., U.S. Dept. Agric., No. 478 (1935).
[3] U.S. Dept. Agric., *ARS*–33–4 (Dec., 1954).

ENTOMOLOGY

Role of Cholesterol in House Fly Reproduction

It is well established that immature insects, including the larvæ of *Musca vicina* Macq., require a dietary source of sterols for growth and development[1,2]. Chauvin[3] reported that sterols are necessary for reproduction in the German cockroach but did not present supporting evidence or describe the effect of sterol deficiency on the reproductive processses. The house fly, which has been found to lack the mechanism for sterol biosynthesis from ^{14}C-sodium acetate[4], utilizes a high percentage of administered ^{14}C-cholesterol in egg production[5]. The following study was undertaken to determine the effect of dietary cholesterol on reproduction of the house fly.

Adult house flies (*Musca domestica* L.) reared by the *CSMA* procedure[6], were allowed to emerge from puparia placed in screen cages, and feed on a synthetic diet consisting of vitamin-free casein, sucrose, sodium oleate, Wesson's salts, zinc chloride, nucleic acids, and a mixture of B vitamins. Cholesterol, when present, was added to the diet at 0·1 per cent. This diet was as good as or better than our Laboratory's standard diet, sucrose-defatted dry milk solids (1:1), when compared as to adult survival, egg production, egg hatch, and larval viability. Eggs were first collected on the sixth day after the end of emergence and then on every other day until nine collections had been made. The eggs obtained at each collection were suspended in water, thoroughly mixed, and duplicate aliquots of approximately 200 eggs were placed on moist filter paper in Petri dishes. The per centage hatch was recorded after 24 hr.

When the flies were fed on the synthetic diet plus cholesterol, the hatch remained fairly constant (82–96 per cent) (Fig. 1). When cholesterol was omitted from the diet, the hatch was initially lower and decreased rapidly until by the fourth egg collection it was only about 5 per cent. If at this time these flies were given the diet plus cholesterol, the hatch increased progressively until by the ninth collection it was 83 per cent. When the larvæ were reared to adults on the *CSMA* medium, only about

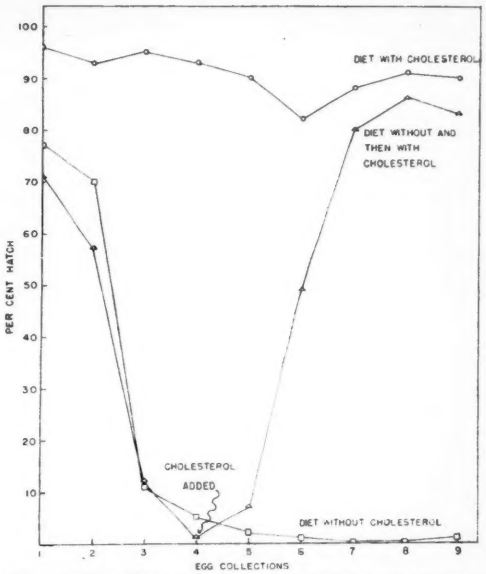

Fig. 1. Effect of dietary cholesterol on egg hatch in the house fly

50 per cent of those that hatched from the sterol-deficient diet became adults as compared with 91 per cent from the diet containing cholesterol.

From these results it appears that a cholesterol-deficient diet not only prevents egg hatch but also inhibits larval development in a medium containing sterols. However, the lack of a dietary sterol had no effect on total egg production.

The full details of this and continued investigations will be published elsewhere.

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[1] Albritton, E. C., 'Standard Values in Nutrition and Metabolism', **28**, 295 (*WADC* Tech. Rep., 1953).
[2] Levinson, Z. H., and Bergmann, E. D., *Biochem. J.*, **65**, 254 (1957).
[3] Chauvin, M. R., *C. R. Acad. Sci., Paris*, **229**, 902 (1949).
[4] Robbins, W. E., Kaplanis, J. N., Louloudes, S. J., and Monroe, R. E., *Ann. Ent. Soc. America* (in the press).
[5] Kaplanis, J. N., Robbins, W. E., and Tabor, L. A. (unpublished work).
[6] Chemical Specialty Manufacturers Association. Peet-Grady Method. 'Soap and Sanitary Chem. Blue Book', **249**, 267 (1955).

BACTERIOLOGY

Difference between the Catalases of Acid-fast Bacilli

It is well known fact that acid-fast bacilli, virulent and rendered resistant to isonicotinic acid hydrazide become catalase-negative. The saprophytic bacilli, on the other hand, grown in even high concentrations of isonicotinic acid hydrazide always preserve at least some residual catalase activity[1,2]. This difference implies that there are different catalases in the above-mentioned strains of acid-fast bacilli.

Our investigations revealed that the catalase of virulent bacilli (*H37Rv*) has both higher activation energy and lower susceptibility to sodium azide, than that of saprophytic bacilli (*M. smegmatis*).

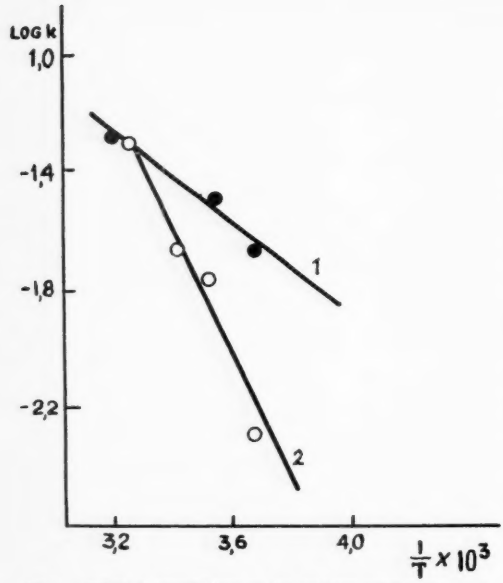

Fig. 1. Effect of temperature on the catalase activity of mycobacteria. Graphic presentation of a typical experiment. Curve 1, suspension of washed cells of *M. smegmatis*. 8-days culture on Sauton medium; curve 2, suspension of washed cells of *M. tub. H37Rv*. 3-weeks culture on Sauton medium

Catalase activity of washed suspensions of cell-free preparations was determined according to the routine iodometric method, slightly modified[3].

Fig. 1 presents the results of the determinations of activation energy. Activation energy for the strain *H37Rv* is 9400 cal. ± 500 *S.D.*; it was calculated from three experiments carried out at various times; at each experiment three or four temperatures ranging from 0° to 40°C. in double samples, were investigated. Five analogous experiments were carried out with *M. smegmatis*; the mean activation energy for this strain is 4300 cal. ± 500.

Sodium azide at a concentration of $1 \times 10^{-6} M$ inhibits catalase activity of the virulent strain by 5 per cent, while the respective value for *M. smegmatis* is 84 per cent. Other inhibitors potassium cyanide, *p*-chlormercuribenzoate and sodium fluoride do not bring about such a difference in acting on these two strains.

Our findings seem to indicate that there are at least two different catalases in acid-fast bacilli. Catalase present in virulent bacilli possesses higher activation energy and is 'azide-resistant' and 'isonicotinic acid hydrazide-susceptible'. The catalase of saprophytic bacilli is 'azide-sensitive' and 'isonicotinic acid hydrazide-resistant'. Both catalases (or two mechanisms of the synthesis of catalase) might be present in saprophytes and therefore these bacilli acquiring resistance to isonicotinic acid hydrazide lose their catalase which is susceptible to isonicotinic acid hydrazide while the resistant catalase remains. Since virulent bacilli possess susceptible catalase exclusively, they lose catalase activity completely, while acquiring resistance to isonicotinic acid hydrazide.

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[1] Bonicke, R., *Naturwiss.*, **41**, 430 (1954).
[2] Kwiek, S. (unpublished experiments).
[3] Tysarowski, W., and Kwiek, S., *Amer. Rev. Tuberc.* (in the press).

Influence of Coagulase on the Growth of Staphylococci in Normal Human Serum

Coagulase-positive staphylococci multiply easily in normal human sera[1] and are not susceptible to the properdin system[2]. In contrast, coagulase-negative strains of these micro-organisms are inhibited in such serum[3]. Free staphylococcal coagulase may be a factor destroying the bactericidal properties of serum[4–6]. Other authors obtained contradictory results[7].

During series of studies on staphylococcal coagulases[8–11] 169 strains of staphylococci excreting large amounts of free coagulase and 28 coagulase-negative populations were observed. Almost all coagulase-positive strains were also hæmolytic, phosphatase- and gelatinase-positive, formed aureus pigment, and fermented mannitol anaerobically. For the study of the influence of serum on staphylococci, 5 kinds of human sera were used, differing in the number of donors and storage. The bacterial count was determined by the duplicate pour plate method.

All coagulase-positive strains grew rapidly in all sera studied. Coagulase-negative strains, however, were inhibited, as illustrated in the Table 1.

Table 1. Growth of Staphylococci in Fresh Human Serum after Incubation for 24 Hr. at 37°

Free coagulase	Bacterial count after hours	Number of viable cells in 1 ml. of serum: less than 400×10^7	400×10^7 – 800×10^7	more than 800×10^7
+	0	164	4	1
+	24	43	58	68
—	0	28	—	—
—	24	28	—	—

Cells of coagulase-negative staphylococci were not always destroyed by serum; in most cases the number of bacteria after 24-hr. incubation was about the same as before incubation.

The kind of storage influences the results. It was found, that only fresh serum possesses full bactericidal activity. Serum frozen for 1 hr. after extraction and stored at $-15°$ loses a part of this activity. It can be used in quantitative determinations, but has bacteriostatic, not bactericidal action on coagulase-negative populations. Serum stored at 4° for 7 days did not have any bacteriostatic or bactericidal activity.

Addition of the cell-free Seitz culture-filtrate of *Staphylococcus J–373* culture[9] containing large amounts of free coagulase enables the growth of coagulase-negative strains (Table 2). The bacterial count, after 24-hr. incubation at 37° in serum, increased with the amount of coagulase added.

Table 2. Influence of Purified Coagulase and Coagulase-containing Culture Filtrate on the Growth of Coagulase Negative Staphylococci in Normal Serum

Number of strains	Amount of added coagulase	Number of viable cells in 1 ml. of serum (24 hr., 37°): less than 400×10^7	400×10^7 – 800×10^7	800×10^7 – 1500×10^7	more than 1500×10^7
25	—	22	3	—	—
25	0·4 ml.*	—	—	17	8
25	0·4 ml.*	—	—	2	23
25	2 mg.†	20	3	2	—
10	2 mg.**	8	1	1	—

* Culture filtrate of Staphylococcus J–373 secreting large amounts of coagulase (ref. 9).
† Purified coagulase (ref. 9).
** Purified coagulase obtained from Prof. M. Tager.

Highly purified preparations obtained from Prof. M. Tager and prepared in this laboratory[9] did not support the growth of coagulase-negative strains.

It was observed that clarity of serum did not exclude the presence of staphylococci. The number of cells up to 500×10^7 in one ml. of serum did not show visible turbidity. A similar phenomenon has been described for 25 per cent albumin solution[12].

According to the results it cannot be concluded that coagulase is a factor impairing the bactericidal activity of human serum. The substance found in culture filtrate of strongly coagulase-positive strain, which stimulates the inhibited growth of coagulase-negative staphylococci, is yet not identified.

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[1] Spink, W. W., Paine, J. R., *J. Immun.*, **38**, 383 (1940).
[2] Pillemer, L., Blum, L., Lepow, I. H., Ross, O. A., Todd, E. W., Wardlaw, A. C., *Science*, **120**, 279 (1954).
[3] Spink, W. W., Vivino, J. J., *J. Clin. Invest.*, **21**, 353 (1942).
[4] Ekstedt, R. D., Nungester, W. J., *Proc. Soc. Exp. biol. and Med.*, **89**, 90 (1955).
[5] Ekstedt, R. D., *J. Bacteriol.*, **72**, 137 (1956).
[6] Ekstedt, R. D., *Ann. N.Y. Acad. Sci.*, **65**, 119 (1956).
[7] Myrvik, Q. N., *Ann. N.Y. Acad. Sci.*, **66**, 391 (1956).
[8] Jeljaszewicz, J., *Postepy Hig. Med. Dośw.*, **10**, 207 (1958).
[9] Jeljaszewicz, J., *Med. Dośw. Mikrobiol.*, **10**, 287 (1958).
[10] Jeljaszewicz, J., *Acta Microbiol. Pol.*, **7**, 17 (1958).
[11] Hasik, J., Jeljaszewicz, J., *Intern. Rev. Phys. Med.*, **3**, 45 (1959).
[12] Wichelhausen, R. H., Clark, W. H., Griffing, V. F., Robinson, L. B., *J. Lab. and Clin. Med.*, **51**, 276 (1958).

PSYCHOLOGY

Experimental Evidence for Extra-Sensory-Perception

The main experimental evidence for extra-sensory-perception produced in Great Britain is that published by S. G. Soal and K. M. Goldney[1] in which they claimed that their subject displayed precognitive telepathy. I have stated elsewhere[2] that the result of this experiment could have been produced by normal means. I now propose to show that this explanation in terms of everyday processes—besides being possible—is necessary in order to account for features which are present in the published results.

The explanation previously suggested depends basically on the subject (or percipient) making pre-arranged 'guesses' at predetermined positions on a printed form. In the experiment, runs of 50 'guesses' were recorded in two columns on this form, each column having spaces for 25 'guesses'. Each fifth line in each column of the form was doubly underlined and numbered. The remaining lines were singly underlined and were not numbered. In Table 1 is shown the

Table 1. Numbers of (+ 2) Hits made at each Position on the Form at which *A* (+ 2) Hit could be Scored

Position	1	2	3	4	5	6	7	8	9	10	11	12
Score	15	22	16	15	11	20	17	14	11	10	14	16
13 14 15 16 17 18 19 20 21 22 23												
24 11 10 12 17 16 14 12 14 18 14												

Total 343

total number of hits made at each of the 25 positions when the two columns on each form are combined. It is based on Table XXXII (p. 139) of the original report[1] and it includes the results of all the experiments carried out at the 'rapid-rate' (about 1·3 sec. per trial) between March 21, 1941, and June 5, 1942. It was in these experiments at the 'rapid-rate' that the subject

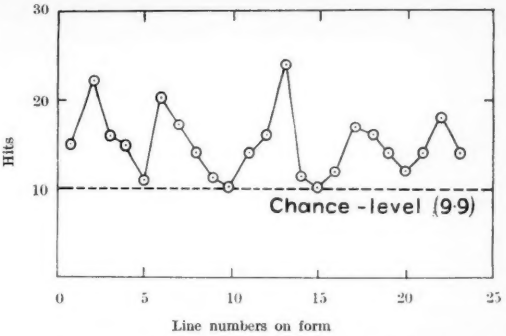

Fig. 1. Scores obtained on the different line numbers of the form on which the subject recorded his 'guesses'

scored significantly above the chance-level, when his 'guesses' were checked against the targets to be seen by a second person two trials ahead (called '+ 2 hits').

The distribution of scores at the different positions in the columns of the form is shown in Fig. 1.

It will be seen that there is a periodicity in the scores which fall to near the chance-level on every fifth line number. When the scores are arranged in groups as in Table 2, we can see how they are affected by their position on the form relative to lines 5, 10, 15, 20.

Table 2. Scores Grouped According to their Positions on the Form in Relation to Lines 5, 10, 15, 20

Position	Lines	Scores	*N*	Total Hits	Mean Scores
$5n^* + 0$	5, 10, 15, 20	11, 10, 10, 12	4	43	10·75
$5n + 1$	1, 6, 11, 16, 21	15, 20, 14, 12, 14	5	75	15·00
$5n + 2$	2, 7, 12, 17, 22	22, 17, 16, 17, 18	5	90	18·00
$5n + 3$	3, 8, 13, 18, 23	16, 14, 24, 16, 14	5	84	16·80
$5n + 4$	4, 9, 14, 19	15, 11, 11, 14	4	51	12·75
		Totals	23	343	

n^* assumes values 0, 1, 2, 3, 4

On some forms the complete run of 50 trials was not completed. The total number of attempts is given as 1,139 and from this figure the number of 'misses' for each of the five groups has been calculated. Table 3 shows the observed numbers of hits and misses and those estimated on the basis of equal scoring ability in each group of positions. From this Table $\chi^2 = 14{\cdot}83$, $n = 4$, $P < 0{\cdot}01$.

Table 3. Observed Numbers of Hits and Misses and Values Estimated on Null Hypothesis

Position	Observed Hits	Observed Misses	Estimated Hits	Estimated Misses
$5n + 0$	43	155·08	59·65	138·43
$5n + 1$	75	172·61	74·57	173·04
$5n + 2$	90	157·61	74·57	173·04
$5n + 3$	84	163·61	74·57	173·04
$5n + 4$	51	147·08	59·65	138·43
Totals	343	795·99	343·01	795·98

Thus it appears that the subject's ability to score in some way depended on the position on the form at which he was recording his 'guess'. It is difficult to explain the periodicity in the scores on the basis of any supposed precognitive ability, but it is quite consistent with the hypothesis that the subject was making pre-arranged 'guesses' at predetermined positions on the form. The periodicity which is present in the figures would arise if these predetermined positions were at fixed distances from the double lines, which were also numbered (lines 5, 10, 15, 20, 25) on the form, and if

the five such possible arrangements were not used equal numbers of times. A fuller investigation of the original data should throw further light on this observation.

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[1] Soal, S. G., and Goldney, K. M., *Proc. Soc. Psych. Res.*, **47**, Pt. 167 (1943).
[2] Hansel, C. E. M., *New Scientist*, **5**, No. 119 (1959).

Some New Subjective Phenomena Apparently Due to Interocular Transfer

There has been a long dispute as to whether visual after-images arise retinally or centrally. An after-image produced in one eye may be seen as if with the other when that alone is open[1]. This transfer of after-images may be due to a persisting central change produced by stimulation of the one eye, or by after-discharge from the stimulated eye being seen as though it arose from the non-stimulated eye. Both mechanisms may be operating but it has never been possible conclusively to dissociate the two possibilities under these conditions.

Craik[2] shone a light into an eye, the optic nerve of which was temporarily paralysed by pressure. When the paralysis was removed an after-image was seen. This is evidence that after-images are retinal in origin, but does not exclude the possibility of them being both central and retinal. I have to report some evidence that after-images may be central in part.

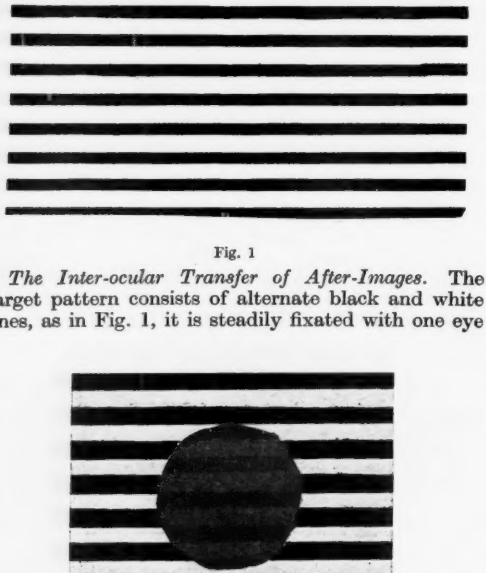

Fig. 1

The Inter-ocular Transfer of After-Images. The target pattern consists of alternate black and white lines, as in Fig. 1, it is steadily fixated with one eye

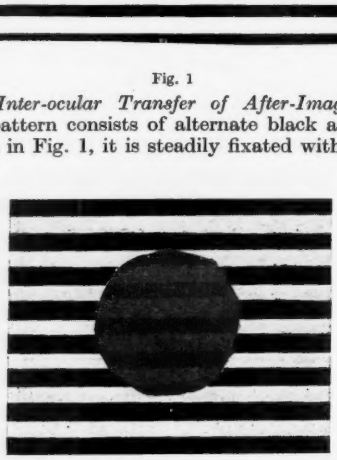

Fig. 2

about 12 in. distant. If one attends to the closed eye, a definite impression of the lines may be seen, apparently with the closed eye, in an area about ½ in. across, roughly the same as the foveal projection. It will be referred to as the 'foveal line after-image'. Where it appears the target lines are blotted out. An impression of the effect is illustrated in Fig. 2. The illusory lines are complementary to the target lines; where there are black lines in the one, there are white lines in the other. The illusion comes and goes as if there were retinal rivalry. It is not to be confused with the simultaneous 'after-image' effect to be seen by the open eye on the target pattern. Care must be taken to avoid forming an after-image of the lines in the eye which is to be closed. Only seven subjects have as yet seen the subjective lines, but their reports were unsolicited. The effect is seen best when the lines are horizontal.

A Lattice Transfer Effect Produced by Viewing Line Patterns. MacKay[3] reported that, when viewing line patterns, the unused eye apparently sees a complementary (at right angles) streaming effect.

Many of our subjects were able to see this effect, but they also saw intermittently, apparently in the closed eye, a diagonally placed lattice pattern, rather like woven basket work, blotting out the line pattern. The target pattern is Fig. 3, made to oscillate slowly.

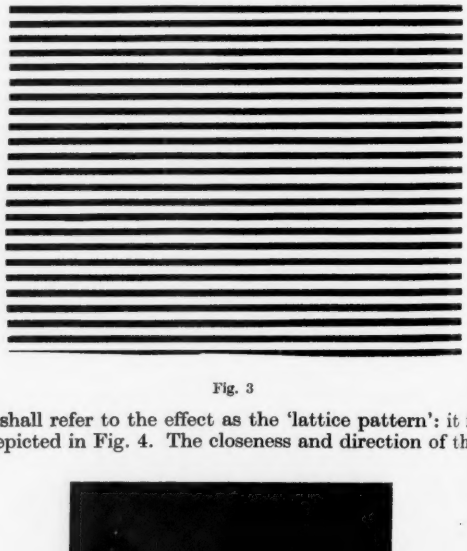

Fig. 3

I shall refer to the effect as the 'lattice pattern': it is depicted in Fig. 4. The closeness and direction of the

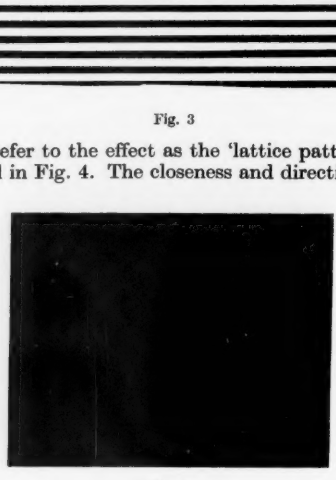

Fig. 4

lattice is related to the closeness and direction of the target pattern lines. It is thus not some entoptic structural feature which is seen. MacKay (personal communication) confirms these observations on the lattice.

From these two observations it is possible to conclude that some central, cortical change is produced by viewing a line target which persists after the binocular rivalry mechanism has suppressed the visual impression of the lines from that eye. It is possible to see a sort of lattice pattern and a line after-image superimposed on the line target but the effects I am reporting occur only when the line target is blotted out by the appearance of the rivalling closed eye field. The following three pieces of evidence further demonstrate that the effects are central and depend upon the activity of the closed eye system.

(1) Seven one-eyed subjects were tested and none of them was able to see the lattice or the foveal line after-image, out of 24 normal subjects tested, 3 could not see the lattice. If one-eyed people do not see these effects, then the effects are dependent on the retina of the covered eye or the central visual areas of one-eyed people are deteriorated: in any case they fail to show any evidence of binocular rivalry.

(2) Further evidence on this point has been gained by doing an experiment suggested by R. L. Gregory. A distinctive and separate after-image was produced in each eye from two differently orientated lamp filaments placed as targets in a stereoscope, prior to viewing the target pattern with one eye. It was seen that the alteration between seeing the target line pattern and the subjective lattice pattern or foveal line effect was related to the alternation between the after-images in the corresponding eyes.

(3) Finally, if a strong flickering light is shone into one eye while the other views the line pattern, the lattice and the foveal lines may be seen very clearly, as if in the flickering light, not now on a dark background, and their form is influenced by the frequency of the flashing light.

Thus, it is reasonable to conclude that these effects are central, probably cortical, in origin, and are seen when and where the closed eye field is dominant. Two things may be happening: either these after-images are induced in the cortical areas of the closed eye and are then seen when that eye is dominant, this would be true binocular transfer, or they are produced in the cortical areas of the open eye and survive the binocular suppression mechanism. If the second explanation were true, then it would suggest that the binocular suppression mechanism is at a level below the site of these after-images, perhaps geniculate.

To decide this issue, one eye was paralysed by pressure while the other viewed the line pattern. Although the dark field of the paralysed eye system could be seen in rivalry with the lines before paralysis was complete, no lattice pattern or foveal line image could be seen. When paralysis was complete there was no rivalry. This suggests either that the closed eye is the site of the lattice effect or that spontaneous afferent discharges from the closed eye are an essential component. The lattice could be an interference pattern produced by interaction of a weak transferred image of the line target (the foveal line after-image) and the spontaneous random activity ascending from the closed eye. This interpretation is supported by evidence which we are at present studying, and which will be presented at a later date.

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[1] Sumner, F. C., and Watts, F. P., *Amer. J. Psychol.*, **48**, 109 (1936).
[2] Craik, K. J. W., *Nature*, **145**, 512 (1940).
[3] MacKay, D. M., *Nature*, **180**, 849 (1957).

Application of Biometrical Genetics to Behaviour in Rats

PREVIOUS work on the psychological genetics of behaviour ('psychogenetics') in animals has usually attempted to analyse major gene effects. Few aspects of behaviour are suitable for such analysis, and the techniques of biometrical genetics, developed by Mather[1] for studying continuous variation, and employing the polygenic hypothesis, were therefore applied to two behavioural characteristics in the rat, namely, the defæcation and ambulation responses observed on exposure to the slight stress of the open-field test, and which are regarded as indices of emotional reactivity[2].

The diallel cross method[3,4], which involves the reciprocal inter-crossing of several strains, was used. It has the advantage for mammalian psychogenetical work that information about genetical parameters is obtained from the $F1$ crosses, without breeding $F2$'s and back-crosses. The six strains used are shown in Table 1. Unlike the other five strains, strain No. 6's

Table 1. ORIGINS AND CHARACTERISTICS OF THE STRAINS USED

No.	Name	Coat-colour Genotype	Obtained from :—	No. generations of inbreeding
1	Albino	*AAcchh*	Agricultural Research Council, Compton.	68–70
2	Black-hooded I	*aaCChh*	ditto	40–43
3	Brown-hooded	*AACChh*	University of Miami, Florida	56–58
4	Black-hooded II	*aaCChh*	ditto	22–23
5	Brown	*AACCHH*	ditto	45–47
6	Maudsley non-reactive	*A?cchh* and *aacchh*	Selective breeding	8

ancestry shows fewer than 20 consecutive generations of brother × sister mating, and consequently cannot be regarded as homozygous. It derives from selection for low defæcation in the open-field test[5] and was included to widen the range of the characteristics studied[6]. During selective breeding, checks showed pre- and post-natal maternal influences to be minimal, and the heritable nature of defæcation and ambulation was effectively demonstrated.

All subjects were reared in a controlled environment with uniform husbandry, and tested in the open field at $100{\cdot}8 \pm 3{\cdot}2$ (standard deviation) days of age. The rats were exposed in the circular arena on four successive days to sound and light fields of uniform intensity at floor level of 78 db. (ref. 0·0002 dynes/sq. cm.), and 165 f.c. respectively. Fæcal boluses deposited constituted the defæcation score, and the number of floor compartments walked through, the ambulation score. For replication purposes, two litters of each cross were bred, and, including representatives of the six parental lines, a total of 72 litters was reared and tested, and the mean scores for each obtained.

In the two 6×6 diallel tables thus generated for each character, the parental values form the leading diagonals, with the crosses and reciprocals above and below. Hayman's analysis[4] was followed, and the resulting variance-covariance diagrams are shown as Fig. 1 (defæcation) and 2 (ambulation). The raw defæcation scores were subjected to a square root transformation to satisfy the first criterion of adequate scaling, the absence of environment-genotype interaction, detected by the presence of significant inhomogeneity of litter variance, and here corrected by the transformation. Another scaling criterion concerns additivity, and departures from it can be detected by significant non-linearity of regression of covariance on variance in the diagram. The covariance of each array in the diallel table with the non-recurrent parent (W_r) is obtained by using the values

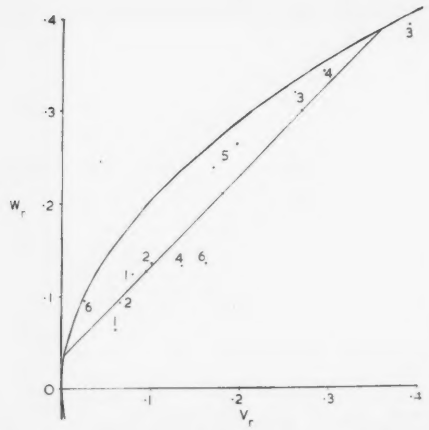

Fig. 1. Variance-covariance diagram for defæcation. The graph shows the regression of W_r on V_r for defæcation scores in the open-field test of a replicated diallel cross of six strains of rats (1–6). Solid points indicate the first litter and open ones the second. The limiting parabola is defined by $W^2_r = V_r.0{\cdot}42$.

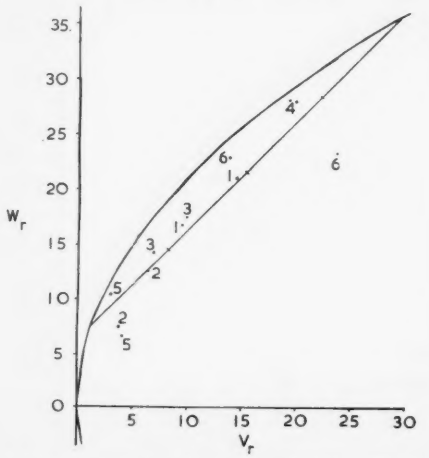

Fig. 2. Variance-covariance diagram for ambulation. The graph shows the regression of W_r on V_r for ambulation scores in the open-field test of a replicated diallel cross of six strains of rats (1–6). Solid points indicate the first litter, and open ones for second. The limiting parabola is defined by $W^2_r = V_r.44{\cdot}3$.

for parental lines in the diagonal, and the array variances (V_r) in the usual way. There were no significant departures from linearity, $b = 0{\cdot}994$ for defæcation (Fig. 1), and $b = 0{\cdot}998$ for ambulation (Fig. 2).

Another important property of the variance-covariance diagram is its indication of average dominance of the genes controlling the character, depending on where the regression line intersects the W_r axis. Both characters show partial or incomplete dominance, somewhat less complete in the case of ambulation, since the intersection is farther above the origin. Division of the regression line into four equal parts between the limiting parabola $W^2_r = V_r.V_1$, where V_1 is the variance of the parental array, indicates an upwards progression of dominants to recessives of 100:0, 75:25, 50:50, etc., respectively. The average dominance order of the six strains is therefore 126543 for defæcation, showing a wide range of ratios of dominants to recessives, and 523164 for ambulation, with no ratio as low as 25:75. Comparing these orders with those for the magnitude of the array means—624153, and 234516, respectively—suggests no preponderance of either increasers or decreasers among dominants or recessives.

Table 2. Components of Variation

Type	Symbol	Characters	
		Defæcation	Ambulation
Heritable Additivity	D	$0{\cdot}26 \pm {\cdot}03$*	$39{\cdot}4 \pm 0{\cdot}7$*
,, Dominance	H_1	$-0{\cdot}15 \pm {\cdot}08$	$6{\cdot}0 \pm 2{\cdot}2$*
,, ,,	H_2	$-0{\cdot}14 \pm {\cdot}07$	$6{\cdot}0 \pm 1{\cdot}9$*†
,, ,,	h^2	$-0{\cdot}07 \pm {\cdot}05$	$1{\cdot}6 \pm 1{\cdot}3$
Non-heritable (Environmental)	E	0·16	4·9

* indicates significant beyond the 5 per cent level, from standard error.
† indicates significant beyond the 5 per cent level, from analysis of variance.

Further analysis of the diallel tables provides estimates of the components of variation for additive and dominant genetical effects (D, and H_1, H_2 and h^2, respectively), and for the non-genetical (environmental) effect (E), with estimates of standard errors for the former. These are given in Table 2, together with estimates of significance obtained independently, and more rigorously, from analyses of variance[7], from which the estimate of E comes, and which also show an absence of significance of the differences between the reciprocal crosses (maternal effects), and between the replications. Among the defæcation data, the large relative value for E indicates a high degree of susceptibility to environmental effects, which precludes further interpretation of the non-significant components. The negative values may be disregarded because they do not differ significantly from zero. The significance of D, however, allows an estimate of heritability—$D/(D+E)$—of 62 per cent. The ambulation data permit the calculation of various parameters in addition to heritability (39 per cent), which suggest that the degree of dominance over all parental loci, $(H_1/D)^{\frac{1}{2}}$, is 0·23, and that the distribution of positive and negative alleles is equal, $H_2/4H_1$ having the maximum value of 0·25. In sum, therefore, it may be suggested that the rat's defæcation in the open field is determined by a polygenic system having a low degree of dominance and a moderate heritability, and more confidently concluded that ambulation is determined by a similar system, also showing a low dominance, but with relatively high heritability.

This experiment demonstrates the applicability of the polygenic hypothesis and the methods of biometrical genetics to problems in psychogenetics. The transition from the physical characteristics of, typically, plants to the behavioural characteristics of mammals has yielded results comparing favourably in ease of analysis and clarity of outcome with those of published diallel crosses.

A full account of this and related work will be published elsewhere[5].

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[1] Mather, K., 'Biometrical Genetics' (Methuen, 1949).
[2] Broadhurst, P. L., *Brit. J. Psychol.*, **48**, 1 (1957).
[3] Jinks, J. L., *Genetics*, **39**, 767 (1954).
[4] Hayman, B. I., *Genetics*, **39**, 789 (1954).
[5] Broadhurst, P. L., in 'Experiments in Personality', **1**, 'Psychogenetics and Psychopharmacology', edit. by Eysenck, H. J. (Routledge and Kegan Paul, in the press).
[6] Broadhurst, P. L., *J. Comp. Physiol. Psychol.*, **51**, 55 (1958).
[7] Hayman, B. I., *Biometrics*, **10**, 235 (1954).